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(54) Title: MAMMALIAN CDR MIMETIBODIES, COMPOSITIONS, METHODS AND USES

(57) Abstract: The present invention relates to at least one novel EPO human CDR mimetibody or specified portion or variant, including isolated nucleic acids that encode at least one CDR mimetibody or specified portion or variant, CDR mimetibody or specified portion or variants, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.



**WO 03/084477 A2**

**MAMMALIAN CDR MIMETIBODIES,  
COMPOSITIONS, METHODS AND USES**

**BACKGROUND OF THE INVENTION**

**FIELD OF THE INVENTION**

5           The present invention relates to mammalian CDR mimetibodies, specified portions and variants specific for biologically active proteins, fragment or ligands, CDR mimetibody encoding and complementary nucleic acids, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

10   **RELATED ART**

          Recombinant proteins are an emerging class of therapeutic agents. Such recombinant therapeutics have engendered advances in protein formulation and chemical modification. Such modifications can potentially enhance the therapeutic utility of therapeutic proteins, such as by increaseing half lives (e.g., by blocking their exposure to proteolytic enzymes), enhancing biological  
15   activity, or reducing unwanted side effects. One such modification is the use of immunoglobulin fragments fused to receptor proteins, such as entercept. Therapeutic proteins have also been constructed using the Fc domain to attempt to provide a longer half-life or to incorporate functions such as Fc receptor binding, protein A binding, and complement fixation.

          One specific and vital role of the mammalian hematopoietic system is the production of  
20   erythrocytes, or red blood cells, which transport oxygen to the various tissues of the animal's body. The process of producing erythrocytes ("erythropoiesis") occurs continuously throughout an animal's life span to offset erythrocyte destruction. The typical red blood cell has a relatively short life-span, usually 100 to 120 days. Erythropoiesis is a precisely controlled physiological mechanism whereby sufficient numbers of erythrocytes are produced to enable proper tissue oxygenation, but not so many  
25   as to impede circulation.

          Erythropoiesis is now known to be primarily controlled by the polypeptide erythropoietin (EPO), an acidic glycoprotein. Erythropoietin is produced as the result of the expression of a single copy gene located in a chromosome of a mammal. The amino acid sequence for recombinant human EPO ("rHuEPO") is substantially identical to the amino acid sequence for EPO obtained from human  
30   urinary sources. However, the glycosylation of rHuEPO differs from that of urinary EPO and human serum EPO.

In a healthy mammal, EPO is present in the blood plasma in very low concentrations, as the tissues are being sufficiently oxygenated by the existing number of circulating erythrocytes. The EPO present stimulates the production of new erythrocytes to replace those lost to the aging process. Additionally, EPO production is stimulated under conditions of hypoxia, wherein the oxygen supply to the body's tissues is reduced below normal physiological levels despite adequate perfusion of the tissue by blood. Hypoxia may be caused by hemorrhaging, radiation-induced erythrocyte destruction, various anemias, high altitude, or long periods of unconsciousness. In contrast, should the number of red blood cells in circulation exceed what is needed for normal tissue oxygenation, EPO production is reduced.

However, certain disease states involve abnormal erythropoiesis. Recombinant human EPO (rHuEPO) is being used therapeutically in a number of countries. In the United States, the U.S. Food and Drug Administration (FDA) has approved rHuEPO's use in treating anemia associated with end-stage renal disease. Patients undergoing hemodialysis to treat this disorder typically suffer severe anemia, caused by the rupture and premature death of erythrocytes as a result of the dialysis treatment. EPO is also useful in the treatment of other types of anemia. For instance, chemotherapy-induced anemia, anemia associated with myelodysplasia, those associated with various congenital disorders, AIDS-related anemia, and prematurity-associated anemia, may be treated with EPO. Additionally, EPO may play a role in other areas, such as helping to more quickly restore a normal hematocrit in bone marrow transplantation patients, in patients preparing for autologous blood transfusions, and in patients suffering from iron overload disorders.

Erythropoietin (EPO) is a glycoprotein hormone composed of 165 amino acids and four carbohydrate chains that functions as the primary regulator of erythropoiesis by binding to a specific receptor on the surface of erythrocyte precursor cells. This binding signals their proliferation and differentiation into mature red blood cells. The erythropoietin receptor is a 484-amino acid glycoprotein with high affinity for erythropoietin. For the erythropoietin receptor, ligand-induced homodimerization is the key event that governs activation .

Erythropoietin has a relatively short half-life. Intravenously administered erythropoietin is eliminated at a rate consistent with first order kinetics with a circulating half-life ranging from approximately 3 to 4 hours in patients with CRF. Within the therapeutic dose range, detectable levels of plasma erythropoietin are maintained for at least 24 hours. After subcutaneous administration of erythropoietin, peak serum levels are achieved within 5-24 hours and decline slowly thereafter. The  $C_{max}$  and  $t_{1/2}$  after administration of erythropoietin were  $1.80 \pm 0.7$  U/mL and  $19.0 \pm 5.9$  hours, respectively.

Starting doses of erythropoietin range from 50-150 U/kg three times weekly. The dosage of erythropoietin must be individualized to maintain the hematocrit within the suggested target range. For

surgery patients the recommended dose of erythropoietin is 300 U/kg/day s.c. for 10 days before surgery, on the day of surgery, and for 4 days after surgery or alternatively 600 U/kg s.c. in once weekly doses (21, 14 and 7 days before surgery) plus a fourth dose on the day of surgery.

Small peptidomimetics of erythropoietin were identified by several groups through screening of random phage display peptide libraries for affinity to the erythropoietin receptor. These sequences have no homology with erythropoietin. In functional assays several of these peptides showed activity, but only 1/100,000<sup>th</sup> that of recombinant erythropoietin. Although several attempts have been made to increase the potency of these peptides by preparing covalent dimers or multimers of peptidomimetics, these compounds are still 1,000 - 10,000 fold less active than erythropoietin on a molar basis.

Peptide sequences from erythropoietin have also been claimed as agonistic. Increased activity of dimerized sequences comprising any or all of the native erythropoietin sequence has also been reported. These compounds have little or no oral bioavailability and their activity does not make them economically viable at this time.

Accordingly, there is a need to provide improved and/or modified versions of therapeutic proteins, which overcome one more of these and other problems known in the art.

### SUMMARY OF THE INVENTION

The present invention provides isolated human mimetibodies, including modified immunoglobulins, cleavage products and other specified portions and variants thereof, as well as CDR mimetibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and/or enabled herein, in combination with what is known in the art.

The present invention also provides at least one isolated CDR mimetibody or specified portion or variant as described herein and/or as known in the art.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding specific mimetibodies or specified portions or variants thereof, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising at least one of said isolated CDR mimetibody nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such CDR mimetibody nucleic acids, vectors and/or host cells.

At least one CDR mimetibody or specified portion or variant of the invention mimics the binding to at least one ligand, or has at least one biological activity of, at least one protein, subunit, fragment, portion or any combination thereof. The at least one CDR mimetibody or specified portion

or variant can comprise at least one protein ligand binding region ("LBR") that is preferably comprised of at least one extracellular, soluble, hydrophillic, external or cytoplasmic portion of said protein, which further comprises at least one amino acid from at least one complementarity determining region (CDR) of a heavy or light chain variable region..

5           The at least one CDR mimetibody or specified portion or variant can optionally comprise at least one LBR comprising at least a specified portion of at least one modified or replaced CDR (e.g., CDR1, CDR2 or CDR3 of the heavy or light chain variable region) and/or at least one framework region. The at least one CDR mimetibody or specified portion or variant amino acid sequence can further optionally comprise at least one specified substitution, insertion or deletion.

10           The present invention also provides at least one isolated CDR mimetibody or specified portion or variant as described herein and/or as known in the art, wherein the CDR mimetibody or specified portion or variant has at least one activity, such as, but not limited to known biological activities. A CDR mimetibody can thus be screened for a corresponding activity according to known methods, such as at least one neutralizing activity towards a protein or fragment thereof.

15           The present invention also provides at least one composition comprising (a) an isolated CDR mimetibody or specified portion or variant encoding nucleic acid and/or CDR mimetibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known methods. The composition can optionally further comprise at least one further compound, protein or composition.

20           The present invention also provides at least one method for expressing at least one CDR mimetibody or specified portion or variant in a host cell, comprising culturing a host cell as described herein and/or as known in the art under conditions wherein at least one CDR mimetibody or specified portion or variant is expressed in detectable and/or recoverable amounts.

25           The present invention further provides at least one CDR mimetibody, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of a(n) anemia; a(n) immune/autoimmune; and/or a(n) cancer/infectious, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art.

30           The present invention further provides at least one CDR mimetibody, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of, at least one immune, cardiovascular, infectious, malignant, and/or neurologic disease in a cell, tissue, organ, animal or patient and/or, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art and/or as described herein.

The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one CDR mimetibody or specified portion or variant, according to the present invention.

The present invention further provides at least one anti-idiotypic antibody to at least one CDR mimetibody of the present invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a CDR mimetibody of the present invention. A CDR mimetibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, and the like.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding at least one CDR mimetibody anti-idiotypic antibody, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said CDR mimetibody anti-idiotypic antibody encoding nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such anti-idiotypic antibody nucleic acids, vectors and/or host cells.

The present invention also provides at least one method for expressing at least one CDR mimetibody, or CDR mimetibody anti-idiotypic antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one CDR mimetibody or anti-idiotypic antibody is expressed in detectable and/or recoverable amounts.

The present invention also provides at least one composition comprising (a) an isolated CDR mimetibody encoding nucleic acid and/or CDR mimetibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known carriers or diluents. The composition can optionally further comprise at least one further compound, protein or composition.

The present invention further provides at least one CDR mimetibody method or composition, for administering a therapeutically effective amount to modulate or treat at least one protein related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one CDR mimetibody, according to the present invention.

The present invention further provides at least one CDR mimetibody method or composition, for diagnosing at least one protein related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

5 The present invention also provides at least one composition, device and/or method of delivery for diagnosing of at least one CDR mimetibody, according to the present invention.

In one aspect, the present invention provides at least one isolated mammalian CDR mimetibody, comprising at least one variable region comprising at least a portion of at least one CDR that further comprises at least one of SEQID NOS:1-1109.

10 In another aspect, the present invention provides at least one isolated mammalian CDR mimetibody, comprising either (i) all of the heavy chain complementarity determining regions (CDR); or (ii) all of the light chain CDR amino acids sequences.

In another aspect, the present invention provides at least one isolated mammalian CDR mimetibody, comprising at least a portion of at least one heavy chain or light chain CDR having inserted therein at least one amino acid sequence of SEQID NOS:1-1110

15 In other aspect the present invention provides at least one isolated mammalian CDR mimetibody, comprising at least one human CDR, wherein the CDR mimetibody specifically binds at least one epitope comprising at least 1-3 of at least one ligand or binding region which ligand binds to at least a portion of at least one of SEQID NOS: 1-1109.

20 The at least one CDR mimetibody can optionally further at least one of: bind protein with an affinity of at least one selected from at least  $10^{-9}$  M, at least  $10^{-10}$  M, at least  $10^{-11}$  M, or at least  $10^{-12}$  M; substantially neutralize at least one activity of at least one protein or portion thereof. Also provided is an isolated nucleic acid encoding at least one isolated mammalian CDR mimetibody; an isolated nucleic acid vector comprising the isolated nucleic acid, and/or a prokaryotic or eukaryotic host cell comprising the isolated nucleic acid. The host cell can optionally be at least one selected from COS-1,  
25 COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof. Also provided is a method for producing at least one CDR mimetibody, comprising translating the CDR mimetibody encoding nucleic acid under conditions in vitro, in vivo or in situ, such that the CDR mimetibody is expressed in detectable or recoverable amounts.

30 Also provided is a composition comprising at least one isolated mammalian CDR mimetibody and at least one pharmaceutically acceptable carrier or diluent. The composition can optionally further comprise an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a

neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

The present invention further provides an anti-idiotypic antibody or fragment that specifically binds at least one isolated mammalian protein antibody of the present invention.

Also provided is a method for diagnosing or treating a protein related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian CDR mimetibody of the invention with, or to, the cell, tissue, organ or animal. The method can optionally further comprise using an effective amount of 0.001-50 mg/kilogram of the cells, tissue, organ or animal. The method can optionally further comprise using the contacting or the administering by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelical, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal. The method can optionally further comprise administering, prior, concurrently or after the (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

Also provided is a medical device, comprising at least one isolated mammalian CDR mimetibody of the invention, wherein the device is suitable to contacting or administering the at least one CDR mimetibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelical, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial,



intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated mammalian CDR mimetibody of the present invention. The article of manufacture can optionally comprise having the container as a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

Also provided is a method for producing at least one isolated mammalian CDR mimetibody of the present invention, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts the CDR mimetibody. Further provided in the present invention is at least one CDR mimetibody produced by the above method.

The present invention also provides at least one method for expressing at least one CDR mimetibody, or anti-idiotypic antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one CDR mimetibody is expressed in detectable and/or recoverable amounts.

The present invention further provides any invention described herein.

## DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic mimetibodies or specified portions or variants, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one CDR mimetibody. Such mimetibodies or specified portions or variants of the present invention comprise specific CDR mimetibody sequences, domains, fragments and specified variants thereof, and methods of making and using said nucleic acids and mimetibodies or specified portions or variants, including therapeutic compositions, methods and devices.

As used herein, a "CDR mimetibody," "CDR mimetibody portion," or "CDR mimetibody fragment" and/or "CDR mimetibody variant" and the like mimics, has or simulates at least one ligand binding or at least one biological activity of at least one protein, such as ligand binding or activity *in vitro*, *in situ* and/or preferably *in vivo*. For example, a suitable CDR mimetibody, specified portion or

variant of the present invention can bind at least one protein ligand and includes at least one protein ligand, receptor, soluble receptor, and the like. A suitable CDR mimetibody, specified portion, or variant can also modulate, increase, modify, activate, at least one protein receptor signaling or other measurable or detectable activity.

5 Mimetibodies useful in the methods and compositions of the present invention are characterized by suitable affinity binding to protein ligands or receptors and optionally and preferably having low toxicity. In particular, a CDR mimetibody, where the individual components, such as the variable region, constant region and framework, or any portion thereof (e.g., a portion of the J, D or V regions of the variable heavy or light chain; the hinge region, the constant heavy chain or light chain, and the like) individually and/or collectively optionally and preferably possess low immunogenicity, is useful in the present invention. The mimetibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, may contribute to the therapeutic results achieved. "Low immunogenicity" is defined  
10 herein as raising significant HAMA, HACA or HAHA responses in less than about 75%, or preferably less than about 50, 45, 40, 35, 30, 35, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, and/or 1 % of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (see, e.g., Elliott *et al.*, *Lancet* 344:1125-1127 (1994)).

#### 20 *Utility*

The isolated nucleic acids of the present invention can be used for production of at least one CDR mimetibody, fragment or specified variant thereof, which can be used to effect in an cell, tissue, organ or animal (including mammals and humans), to modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one protein related condition, selected from, but not limited to, at least one of an immune disorder or disease, a cardiovascular disorder or disease, an infectious,  
25 malignant, and/or neurologic disorder or disease, a(n) anemia; a(n) immune/autoimmune; and/or a(n) cancer/infectious, as well as other known or specified protein related conditions.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to  
30 a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.0001 to 500 mg/kg per single or multiple administration, or to achieve a serum concentration of 0.001-5000 µg/ml serum concentration per single or multiple administration, or any effective range or

value therein, as done and determined using known methods, as described herein or known in the relevant arts.

### *Citations*

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2000); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2000); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2000).

### *Mimetibodies of the Present Invention*

The term "CDR mimetibody" is intended to encompass any antibody fragment where a protein is inserted into, or replaces a portion of, one or more CDR regions (e.g., CDR1, CDR2 or CDR3, heavy chain or light chain), the combination of the protein and the CDR or portion thereof designated a ligand binding region (LBR) of the CDR mimetibody. Such mimetibodies include those that mimic the structure and/or function of at least one protein or fragment or analog. Functional portions of at least one CDR mimetibody of the present invention include at least one ligand- or antigen-binding fragment that binds to at least one protein ligand or receptor. For example, CDR mimetibody fragments capable of binding to human ligands or portions thereof, including, but not limited to mimetics of Fv, Fab, Fab' and F(ab')<sub>2</sub> fragments, and any other portion of an antibody, further comprising at least one ligand binding region (LBR) are encompassed by the present invention.

In particular, mimetibodies comprise at least one ligand binding region (LBR) that corresponds to at least one portion of at least one complementarity determining region (CDR, e.g., CDR1, CDR2 or CDR3 of HC or LC variable region) of at least one antibody or fragment or portion thereof where at least one ligand protein is inserted into or replaces at least a portion of at least one CDR of the antibody or portion thereof. Such mimetibodies of the present invention thus provide at least one suitable property as compared to known proteins, such as, but not limited to, at least one of increased half-life, increased activity, more specific activity, increased avidity, increased or decreased off rate, a selected or more suitable subset of activities, less immunogenicity, increased quality or duration of at least one desired therapeutic effect, less side effects, and the like.

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. For example, papain or pepsin cleavage can generate CDR mimetibody Fab or F(ab')<sub>2</sub> fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab or F(ab')<sub>2</sub> fragments or portions thereof. Mimetibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab')<sub>2</sub> heavy chain portion can be designed to include DNA sequences encoding the CH<sub>1</sub> domain and/or hinge region of the heavy chain. The various portions of mimetibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, a nucleic acid encoding the variable and constant regions of a human antibody chain can be expressed to produce a contiguous protein for use in mimetibodies of the present invention. See, e.g., Ladner *et al.*, U.S. Patent No. 4,946,778 and Bird, R.E. *et al.*, *Science*, 242: 423-426 (1988), regarding single chain mimetibodies.

As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., LBR, framework, C<sub>L</sub>, C<sub>H</sub> domains (e.g., C<sub>H</sub>1, C<sub>H</sub>2, C<sub>H</sub>3), hinge, (V<sub>L</sub>, V<sub>H</sub>)) is substantially non-immunogenic, with only minor sequence changes or variations. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans relative to non-modified human antibodies, or mimetibodies of the present invention. Thus, a human antibody and corresponding CDR mimetibody of the present invention is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody and CDR mimetibody can be produced by a non-human animal or cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes, and for a CDR mimetibody, wherein at least one Ig CDR is replaced by an LBR of at least one ligand protein or fragment.

Human mimetibodies that are specific for at least one protein ligand or receptor thereof can be designed against an appropriate ligand, such as isolated and/or protein receptor or ligand, or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of such mimetibodies are performed using known techniques to identify and characterize ligand binding regions or sequences of at least one protein or portion thereof.

In a preferred embodiment, at least one CDR mimetibody or specified portion or variant of the present invention is produced by at least one cell line, mixed cell line, immortalized cell or clonal population of immortalized and/or cultured cells. Immortalized protein producing cells can be produced using suitable methods. Preferably, the at least one CDR mimetibody or specified portion or variant is generated by providing nucleic acid or vectors comprising DNA derived or having a substantially similar sequence to, at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement, and which further comprises at least one

CDR that has been replaced by at least one LBR specific for at least one protein ligand, the resulting nucleic acid encoding at least one CDR mimetibody, or specified portion or variant according to the present invention.

The term "functionally rearranged," as used herein refers to a segment of nucleic acid from an immunoglobulin locus that has undergone V(D)J recombination, thereby producing an immunoglobulin gene that encodes an immunoglobulin chain (e.g., heavy chain, light chain), or any portion thereof. A functionally rearranged immunoglobulin gene can be directly or indirectly identified using suitable methods, such as, for example, nucleotide sequencing, hybridization (e.g., Southern blotting, Northern blotting) using probes that can anneal to coding joints between gene segments or enzymatic amplification of immunoglobulin genes (e.g., polymerase chain reaction) with primers that can anneal to coding joints between gene segments. Whether a cell produces an CDR mimetibody or portion or variant comprising a particular variable region or a variable region comprising a particular sequence (e.g., at least one ligand binding region (LBR) sequence) can also be determined using suitable methods.

Mimetibodies, specified portions and variants of the present invention can also be prepared using at least one CDR mimetibody or specified portion or variant encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such mimetibodies or specified portions or variants in their milk. Such animals can be provided using known methods as applied for antibody encoding sequences. See, e.g., but not limited to, US patent nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Mimetibodies, specified portions and variants of the present invention can additionally be prepared using at least one CDR mimetibody or specified portion or variant encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such mimetibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., Curr. Top. Microbol. Immunol. 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. Antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain mimetibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101-109 (1998) and references cited therein. Thus, mimetibodies, specified portions and variants of the present invention can also be

produced using transgenic plants, according to known methods. See also, e.g., Fischer et al., *Biotechnol. Appl. Biochem.* 30:99-108 (Oct., 1999), Ma et al., *Trends Biotechnol.* 13:522-7 (1995); Ma et al., *Plant Physiol.* 109:341-6 (1995); Whitelam et al., *Biochem. Soc. Trans.* 22:940-944 (1994); and references cited therein. The above references are entirely incorporated herein by reference.

5           The mimetibodies of the invention can bind human protein ligands with a wide range of affinities ( $K_D$ ). In a preferred embodiment, at least one human CDR mimetibody of the present invention can optionally bind at least one protein ligand with high affinity. For example, at least one CDR mimetibody of the present invention can bind at least one protein ligand with a  $K_D$  equal to or less than about  $10^{-9}$  M or, more preferably, with a  $K_D$  equal to or less than about 0.1-9.9 (or any range or value therein)  $\times 10^{-10}$  M,  $10^{-11}$ ,  $10^{-12}$ ,  $10^{-13}$  or any range or value therein.

10           The affinity or avidity of a CDR mimetibody for at least one protein ligand can be determined experimentally using any suitable method, e.g., as used for determining antibody-antigen binding affinity or avidity. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In *Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular CDR mimetibody-ligand interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other ligand-binding parameters (e.g.,  $K_D$ ,  $K_a$ ,  $K_d$ ) are preferably made with standardized solutions of CDR mimetibody and ligand, and a standardized buffer, such as the buffer described herein.

## 20   **Nucleic Acid Molecules**

          Using the information provided herein, such as the nucleotide sequences encoding at least 90-100% of the contiguous amino acids of at least one of SEQID NOS:1-1009 as well as at least one portion of an antibody, wherein the above sequences are inserted into or replace at least one CDR of at least one antibody to provide a CDR mimetibody of the present invention, further comprising specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one CDR mimetibody or specified portion or variant can be obtained using methods described herein or as known in the art.

30           Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combination thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to, at least one specified portion of at least one LBR, as LBR1, LBR2 and/or LBR3 of at least one heavy chain or light chain; nucleic acid molecules comprising the coding sequence for a CDR mimetibody or specified portion or variant; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one CDR mimetibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific CDR mimetibody or specified portion or variants of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention.

In another aspect, the invention provides isolated nucleic acid molecules encoding a(n) CDR mimetibody or specified portion or variant having an amino acid sequence as encoded by the nucleic acid contained in the plasmid deposited as designated clone names

\_\_\_\_\_ and ATCC Deposit Nos.  
\_\_\_\_\_, respectively, deposited on  
\_\_\_\_\_.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding a CDR mimetibody or specified portion or variant can include, but are not limited to, those encoding the amino acid sequence of a CDR mimetibody fragment, by itself; the coding sequence for the entire CDR mimetibody or a portion thereof; the coding sequence for a CDR mimetibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding a CDR mimetibody or specified portion or variant can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused CDR mimetibody or specified portion or variant comprising a CDR mimetibody fragment or portion.

#### **Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein**

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein, or others disclosed herein, including specified variants or

portions thereof. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides.

Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 40-99% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of a CDR mimetibody or specified portion or variant encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding a CDR mimetibody or specified portion or variant of the present invention. See, e.g., Ausubel, *supra*; Colligan, *supra*, each entirely incorporated herein by reference.

#### **Construction of Nucleic Acids**

The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. See, e.g., Ausubel, *supra*; or Sambrook, *supra*.

#### **Recombinant Methods for Constructing Nucleic Acids**

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under suitable stringency conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*).



**Synthetic Methods for Constructing Nucleic Acids**

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

**Recombinant Expression Cassettes**

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding a CDR mimetibody or specified portion or variant of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* or *in vitro* by mutation, deletion and/or substitution, as known in the art. A polynucleotide of the present invention can be expressed in either sense or anti-sense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable characteristics. Another method of suppression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to block the transcription of target genes.

**Vectors And Host Cells**

The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one CDR mimetibody or specified portion or variant by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced into a cell using suitable known methods, such as electroporation and the like, other known methods include the use of the vector as a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites optionally for at least one of transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat.Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017, ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, US Pat.Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

At least one CDR mimetibody or specified portion or variant of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of a CDR mimetibody or specified portion or variant to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a CDR mimetibody or specified portion or variant of the present invention to facilitate purification. Such regions can be removed prior to final preparation of a CDR mimetibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

Illustrative of cell cultures useful for the production of the mimetibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, hepG2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (US Pat.Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (US Pat.No. 5,266,491), at least one human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas ([www.atcc.org](http://www.atcc.org)) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

#### **Purification of an CDR mimetibody or specified portion or variant Thereof**

A CDR mimetibody or specified portion or variant can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,

hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2000), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

5 Mimetibodies or specified portions or variants of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the CDR mimetibody or specified portion or variant of the present invention can be glycosylated or can be non-  
10 glycosylated, with glycosylated preferred. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely incorporated herein by reference.

#### **MIMETIBODIES, SPECIFIED FRAGMENTS AND/OR VARIANTS**

The isolated mimetibodies of the present invention comprise a CDR mimetibody or specified  
15 portion or variant encoded by any one of the polynucleotides of the present invention as discussed more fully herein, or any isolated or prepared CDR mimetibody or specified portion or variant thereof.

Preferably, the CDR mimetibody or ligand-binding portion or variant binds at least one protein ligand or receptor, and, thereby provides at least one biological activity of the corresponding protein or a fragment thereof. Different therapeutically or diagnostically significant proteins are well known in the  
20 art and suitable assays or biological activities of such proteins are also well known in the art. Peptides. Any number of peptides may be used in conjunction with the present invention.

Non limiting examples can be

Of particular interest are peptides that mimic the activity of EPO, TPO, growth hormone, G-CSF, GM-CSF, IL-1ra, leptin, CTLA4, TRAIL, TGF- $\alpha$ , and TGF- $\beta$ . Peptide antagonists are also of interest,  
25 particularly those antagonistic to the activity of TNF, leptin, any of the interleukins (IL-1 – IL-23, etc.), and proteins involved in complement activation (e.g., C3b). Targeting peptides are also of interest, including tumor-homing peptides, membrane-transporting peptides, and the like. All of these classes of peptides may be discovered by methods described in the references cited in this specification and other references.

30 A particularly preferred group of peptides are those that bind to cytokine receptors. Cytokines have recently been classified according to their receptor code. See Inglot (1997), Archivum Immunologiae e Therapiae Experimentalis 45: 353-7, which is hereby incorporated entirely by reference.

Non-limiting examples of suitable peptides for this invention appear in Tables 1

through 17 below. These peptides may be prepared by methods disclosed and/or known in the art. Single letter amino acid abbreviations are used in most cases. The X in these sequences (and throughout this specification, unless specified otherwise in a particular instance) means that any of the 20 naturally occurring amino acid residues or know derivatives thereof may be present, or any know modified amino acid thereof. Any of these peptides may be linked in tandem (i.e., sequentially), with or without linkers, and a few tandemlinked examples are provided in the table. Linkers are listed as "Δ" and may be any of the linkers described herein. Tandem repeats and linkers are shown separated by dashes for clarity. Any peptide containing a cysteinyl residue may optionally be cross-linked with another Cys-containing peptide, either or both of which may be linked to a vehicle. A few crosslinked examples are provided in the table. Any peptide having more than one Cys residue may form an intrapeptide disulfide bond, as well; see, for example, EPO-mimetic peptides in Table 1. A few examples of intrapeptide disulfide-bonded peptides are specified in the table. Any of these peptides may be derivatized as described herein, and a few derivatized examples are provided in the table. For derivatives in which the carboxyl terminus may be capped with an amino group, the capping amino group is shown as -NH<sub>2</sub>. For derivatives in which amino acid residues are substituted by moieties other than amino acid residues, the substitutions are denoted by a δ, which signifies any of the moieties known in the art, e.g., as described in Bhatnagar et al. (1996), J. Med. Chem. 39: 3814-9 and Cuthbertson et al. (1997), J. Med. Chem. 40:2876-82, which are entirely incorporated by reference. The J substituent and the Z substituents (Z<sub>5</sub>, Z<sub>6</sub>, ... Z<sub>40</sub>) are as defined in U.S. Pat. Nos. 5,608,035, 5,786,331, and 5,880,096, which are entirely incorporated herein by reference. For the EPO-mimetic sequences (Table 1), the substituents X<sub>2</sub> through X<sub>11</sub> and the integer "n" are as defined in WO 96/40772, which is entirely incorporated by reference. The substituents "Ψ", "Θ", and "+" are as defined in Sparks et al. (1996), Proc. Natl. Acad. Sci. 93: 1540-4, which is entirely incorporated by reference. X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, and X<sub>7</sub> are as defined in U.S. Pat. No. 5,773,569, which is hereby entirely incorporated by reference, except that: for integrin-binding peptides, X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>7</sub>, and X<sub>8</sub> (Table 6), are as defined in PCT applications WO 95/14714, published June 1, 1995 and WO 97/08203, published March 6, 1997, which are also entirely incorporated by reference; and for VIP-mimetic peptides (Table 9), X<sub>1</sub>, X<sub>1</sub>', X<sub>1</sub>'', X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, and Z; and the integers m and n are as defined in WO 97/40070, published October 30, 1997, which is also entirely incorporated herein by reference. Xaa and Yaa below are as defined in WO 98/09985, published March 12, 1998, which is entirely incorporated herein by reference. AA<sub>1</sub>, AA<sub>2</sub>, AB<sub>1</sub>, AB<sub>2</sub>, and AC are as defined in International application WO 98/53842, published December 3,

1998, which is entirely incorporated by reference. X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, and X<sup>4</sup> in Table 14 only are as, defined in European application EP 0 911 393, published April 28, 1999, entirely incorporated herein by reference. Residues appearing in boldface are D-amino acids, but can be optionally L-amino acids. All peptides are linked through peptide bonds unless otherwise noted.

5 Abbreviations are listed at the end of this specification. In the "SEQID NO." column, "NR" means that no sequence listing is required for the given sequence.

**Table 1-EPO-mimetic peptide sequences**

	<u>Sequence/structure;</u>	<u>SEQID NO:</u>
	YXCXXGPXTWXCXP	1
10	YXCXXGPXTWXCXP-YXCXXGPXTWXCXP	2
	YXCXXGPXTWXCXP-A-YXCXXGPXTWXCXP	3
	YXCXXGPXTWXCXP-Δ-ε-amine)	4
	\	
	K	
15	/	
	YXCXXGPXTWXCXP-Δ- (α-amine)	4
	GGTYSCHFGPLTWVCKPQGG	5
	GGDYHCRMGPLTWVCKPLGG	6
	GGVYACRMGPITWVCSPLGG	7
20	VGNYMCHFGPITWVCRPGGG	8
	GGLYLGRFGPVTWDCGYKGG	9
	GGTYSCHFGPLTWVCKPQGG-	10
	GGTYSCHFGPLTWVCKPQGG -Δ-GGTYSCHFGPLTWVCKPQGG	11
	GGTYSCHFGPLTWVCKPQGGSSK	12
25	GGTYSCHFGPLTWVCKPQGGSSK	13
	GGTYSCHFGPLTWVCKPQGGSSK	14
	GGTYSCHFGPLTWVCKPQGGSSK-Δ-GGTYSCHFGPLTWVCKPQGGSSK	
	GGTYSCHFGPLTWVCKPQGGSS -Δ-ε-amine)	
	\	
30	K	
	/	
	GGTYSCHFGPLTWVCKPQGGSS-Δ- (α-amine)	15
	GGTYSCHFGPLTWVCKPQGGSSK(-Δ-biotin)	16
	CX <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> C	17

	GGTYSCHGPLTWVCKPQGG	18
	VGNYMAHMGPIWVCRPGG	19
	GGPHHVYACRMGPLTWIC	20
	GGTYSCHFGPLTWVCKPQ	21
5	GGLYACHMGPMTWVCQPLRG	22
	TIAQYICYMGPETWECRPSKA	23
	YSCHFGPLTWVCK	24
	YCHFGPLTWVC	25
	X <sub>3</sub> X <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> X <sub>8</sub>	26
10	YX <sub>2</sub> X <sub>3</sub> X <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> X <sub>8</sub>	27
	X <sub>1</sub> YX <sub>2</sub> X <sub>3</sub> X <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> X <sub>7</sub> X <sub>8</sub> X <sub>9</sub> X <sub>10</sub> X <sub>11</sub>	28
	X <sub>1</sub> YX <sub>2</sub> CX <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> CX <sub>9</sub> X <sub>10</sub> X <sub>11</sub>	29
	GGLYLRCRFGPVTWDCGYKGG	30
	GGTYSCHFGPLTWVCKPQGG	31
15	GGDYHCRMGPLTWVCKPLGG	32
	VGNYMCHFGPIWVCRPGGG	33
	GGVYACRMGPITWVCSPLGG	34
	VGNYMAHMGPIWVCRPGG	35
	GGTYSCHFGPLTWVCKPQ	36
20	GGLYACHMGPMT%AIVCQPLRG	37
	TIAQYICYMGPETWECRPSKA	38
	YSCHFGPLTWVCK	39
	YCHFGPLTWVC	40
	SCHFGPLTWVCK	41
25	(AX <sub>2</sub> ) <sub>n</sub> X <sub>3</sub> X <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> X <sub>8</sub>	42

Table 2-IL-1 antagonist peptide sequences

	<u>SEQUENCE/STRUCTURE</u>	<u>SEQ ID NO:</u>
	Z <sub>11</sub> Z <sub>7</sub> Z <sub>8</sub> ZQZ <sub>5</sub> YZ <sub>6</sub> Z <sub>9</sub> Z <sub>10</sub>	43
30	XXQZ <sub>5</sub> YZ <sub>6</sub> XX	44
	Z <sub>7</sub> XQZ <sub>5</sub> YZ <sub>6</sub> XX	45
	Z <sub>7</sub> Z <sub>8</sub> QZ <sub>5</sub> YZ <sub>6</sub> Z <sub>9</sub> Z <sub>10</sub>	46
	Z <sub>11</sub> Z <sub>7</sub> Z <sub>8</sub> QZ <sub>5</sub> YZ <sub>6</sub> Z <sub>9</sub> Z <sub>10</sub>	47
	Z <sub>12</sub> Z <sub>13</sub> Z <sub>14</sub> Z <sub>15</sub> Z <sub>16</sub> Z <sub>17</sub> Z <sub>18</sub> Z <sub>19</sub> Z <sub>20</sub> Z <sub>21</sub> Z <sub>22</sub> Z <sub>11</sub> Z <sub>7</sub> Z <sub>8</sub> QZ <sub>5</sub> YZ <sub>6</sub> Z <sub>9</sub> Z <sub>10</sub> L	48

	Z <sub>23</sub> NZ <sub>24</sub> Z <sub>39</sub> Z <sub>25</sub> Z <sub>26</sub> Z <sub>27</sub> Z <sub>28</sub> Z <sub>29</sub> Z <sub>30</sub> Z <sub>40</sub>	49
	TANVSSFEWTPYYWQPYPALPL	50
	SWTDYGYWQPYPALPISGL	51
	ETPFTWEESNAYYWQPYPALPL	52
5	ENTYSPN <del>W</del> ADSMYWQPYPALPL	53
	SVGEDHNFWTSEYWQPYPALPL	54
	DGYDRWRQSGERYWQPYPALPL	55
	FEWTPGYWQPY	56
	FEWTPGYWQHY	57
10	FEWTPGWYQJY	58
	AcFEWTPGWYQJY	59
	FEVffPGW <sub>p</sub> YQJY	60
	FAWTPGYWQJY	61
	FEWAPGYWQJY	62
15	FEWVPGYWQJY	63
	FEWTPGYWQJY	64
	AcFEWTPGYWQJY	65
	FEWTPaWYQJY	66
	FEWTPSarWYQJY	67
20	FEWTPGYYQPY	68
	FEWTPGWWQPY	69
	FEWTPNYWQPY	70
	FEVffP <sub>v</sub> YWQJY	71
	FEWTPecGYWQJY	72
25	FEWTPAibYWQJY	73
	FEVffSarGYWQJY	74
	FEWTPGYWQPY	75
	FEWTPGYWQHY	76
	FEWTPGWYQJY	77
30	AcFEWTPGWYQJY	78
	FEWTPGW-pY-QJY	79
	FAWTPGYWQJY	80
	FEWAPGYWQJY	81
	FEWVPGYWQJY	82
35	FEWTPGYWQJY	83



	AcFEWTPGYWQJY	84
	FEWTPAWYQJY	85
	FEWTPSarWYQJY	86
	FEWTPGYYQPY	87
5	FEWTPGWWQPY	88
	FEWTPNYWQPY	89
	FEWTPVYWQJY	90
	FEWTPecGYWQJY	91
	FEWTPAibYWQJY	92
10	FEWTSarGYWQJY	93
	FEWTPGYWQPYALPL	94
	NapEWTPGYYQJY	95
	YEWTPGYYQJY	96
	FEWVPGYYQJY	97
15	FEWTPSYQJY	99
	FEWTPNYYQJY	99
	TKPR	100
	RKSSK	101
	RKQDK	102
20	NRKQDK	103
	RKQDKR	104
	ENRKQDKRF	105
	VTKFYF	106
	VTKFY	107
25	VTDFY	108
	SHLYWQPYSVQ	109
	TLVYWQPYSLQT	110
	RGDYWQPYSVQS	111
	VHVVYWQPYSVQT	112
30	RLVYWQPYSVQT	113
	SRVWFQPYSLQS	114
	NMVYWQPYSIQT	115
	SVVFWQPYSVQT	116
	TFVYWQPYALPL	117
35	TLVYWQPYSIQR	118

	RLVYWQPYSVQR	119
	SPVFWQPYSIQI	120
	WIEWWQPYSVQS	121
	SLIYWQPYSLQM	122
5	TRLYWQPYSVQR	123
	RCDYWQPYSVQT	124
	MRVFWQPYSVQN	125
	KIVYWQPYSVQT	126
	RHLYWQPYSVQR	127
10	ALVWWQPYSEI	128
	SRVWFQPYSLQS	129
	WEQPYALPLE	130
	QLVWWQPYSVQR	131
	DLRYWQPYSVQV	132
15	ELVWWQPYSLQL	133
	DLVWWQPYSVQW	134
	NGNYWQPYSFQV	135
	ELVYWQPYSIQR	136
	ELMY)AIQPYSVQE	137
20	NLLYWQPYSMQD	138
	GYEWYQPYSVQR	139
	SRVWYQPYSVQR	140
	LSEQYQPYSVQR	141
	GGGWWQPYSVQR	142
25	VGRWYQPYSVQR	143
	VHVYWQPYSVQR	144
	QARWYQPYSVQR	145
	VHVYWQPYSVQT	146
	RSVYWQPYSVQR	147
30	TRVWFQPYSVQR	148
	GRIWFQPYSVQR	149
	GRVWFQPYSVQR	150
	ARTWYQPYSVQR	151
	ARVWWQPYSVQM	152
35	RLMFYQPYSVQR	153

	ESMWYQPYSVQR	154
	HFGWWQPYSVHM	155
	ARFWWQPYSVQR	156
	RLVYWQPYAPIY	157
5	RLVYWQPYSYQT	158
	RLVYWQPYSLPI	159
	RLVYWQPYSVQA	160
	SRVWYQPYAKGL	161
	SRVWYQPYAQGL	162
10	SRVWYQPYAMPL	163
	SRVWYQPYSVQA	164
	SRVWYQPYSLGL	165
	SRVWYQPYAREL	166
	SRVWYQPYSRQP	167
15	SRVWYQPYFVQP	168
	EYEWYQPYALPL	169
	IPEYWQPYALPL	170
	SRIWWQPYALPL	171
	DPLFWQPYALPL	172
20	SRQWVQPYALPL	173
	IRSWWQPYALPL	174
	RGYWQPYALPL	175
	RLLWVQPYALPL	176
	EYRWFQPYALPL	177
25	DAYWVQPYALPL	178
	WSGYFQPYALPL	179
	NIEFWQPYALPL	180
	TRDWVQPYALPL	181
	DSSWYQPYALPL	182
30	IGNWYQPYALPL	183
	NLRWDQPYALPL	184
	LPEFWQPYALPL	185
	DSYWWQPYALPL	186
	RSQYYQPYALPL	187
35	ARFWLQPYALPL	188

	NSYFWQPYALPL	189
	RFMYWQPYSVQR	190
	AHLFWQPYSVQR	191
	WWQPYALPL	192
5	YYQPYALPL	193
	YFQPYALGL	194
	YWYQPYALPL	195
	RWWQPYATPL	196
	GWYQPYALGF	197
10	YWYQPYALGL	198
	IWYQPYAMPL	199
	SNMQPYQRLS	200
	TFVYWQPYAVGLPAAETACN	201
	TFVYWQPYSVQMTITGKVTM	202
15	TFVYWQPYSSHXXVPXGFPL	203
	TFVYWQPYYGNPQWAIHVRH	204
	TFVYWQPYVLLELPEGAVRA	205
	TFVYWQPYVDYVWPIPIAQV	206
	GWYQPYVDGWR	207
20	RWEQPYVKDGWS	208
	EWYQPYALGWAR	209
	GWWQPYARGL	210
	LFEQPYAKALGL	211
	GWEQPYARGLAG	212
25	AWVQPYATPLDE	213
	MWYQPYSSQPAE	214
	GWTQPYSQQGEV	215
	DWFQPYSIQSDE	216
	PWIQPYARGFG	217
30	RPLYWQPYSVQV	218
	TLIYWQPYSVQI	219
	RFDYWQPYSQDT	220
	WHQFVQPYALPL	221
	EWDSVYWQPYSVQTLLR	223
35	WEQNVYWQPYSVQSFAD	224

	SDVVYWQPYSVQSLEM	225
	YYDGVYWQPYSVQVMPA	226
	SDIWYQPYALPL	227
	QRIWWQPYALPL	228
5	SRIWWQPYALPL	229
	RSLYWQPYALPL	230
	TIIWEQPYALPL	231
	WETWYQPYALPL	232
	SYDWEQPYALPL	233
10	SRIWCQPYALPL	234
	EIMFWQPYALPL	235
	DYVWQQPYALPL	236
	MDLLVQWYQPYALPL	237
	GSKVILWYQPYALPL	238
15	RQGANIWYQPYALPL	239
	GGGDEPWWYQPYALPL	240
	SQLERTWYQPYALPL	241
	ETWVREWWYQPYALPL	242
	KKGSTQWYQPYALPL	243
20	LQARMNWWYQPYALPL	244
	EPRSQKWWYQPYALPL	245
	VKQKWRWWYQPYALPL	246
	LRRHDVWWYQPYALPL	247
	RSTASIWWYQPYALPL	248
25	ESKEDQWWYQPYALPL	249
	EGLTMKWWYQPYALPL	250
	EGSREGWWYQPYALPL	251
	VIEWWWQPYALPL	252
	VWYWEQPYALPL	253
30	ASEWWQPYALPL	254
	FYEWQPYALPL	255
	EGWWVQPYALPL	256
	WGEWLQPYALPL	257
	DYVWEQPYALPL	258
35	AHTWWQPYALPL	259

	FIEWFQPYALPL	260
	WLAWEQPYALPL	261
	VMEWWQPYALPL	262
	ERMWQPYALPL	263
5	NXXWXXPYALPL	264
	WGNWYQPYALPL	265
	TLYWEQPYALPL	266
	VWRWEQPYALPL	267
	LLWTQPYALPL	268
10	SRIWXX PYALPL	269
	SDIWYQPYALPL	270
	WGYXX PYALPL	271
	TSGWYQPYALPL	272
	VHPYXXPYALPL	273
15	EHSYFQPYALPL	274
	XXIWYQPYALPL	275
	AQLHSQPYALPL	276
	WANWFQPYALPL	277
	SRLYSQPYALPL	278
20	GVTFSQPYALPL	279
	SIVWSQPYALPL	280
	SRDLVQPYALPL	281
	HWGHVYWQPYSVQDDL	282
	SWHSVYWQPYSVQSVPE	283
25	WRDSVYWQPYSVQPESA	284
	TWDAVYWQPYSVQKWLD	285
	TPPWVYWQPYSVQSLDP	286
	YWSSVYWQPYSVQSVHS	287
	YWYQPYALGL	288
30	YWYQPYALPL	289
	EWIQPYATGL	290
	NWEQPYAKPL	291
	AFYQPYALPL	292
	FLYQPYALPL	293
35	VCKQPYLEWC	294

	ETPFTWEESNAYYWQPYALPL	295
	QGWLTWQDSVDMYWQPYALPL	296
	FSEAGYTPENTYWQPYALPL	297
	TESPGGLDWAKIYWQPYALPL	298
5	DGYDRWRQSGERYWQPYALPL	299
	TANVSSFEWTPGYWQPYALPL	300
	SVGEDHNFWTSE YWQPYALPL	301
	MNDQTSEVSTFPYWQPYALPL	302
	SWSEAFEQPRNLYWQPYALPL	303
10	QYAEPSALNDWGYWQPYALPL	304
	NGDWATADWSNYYWQPYALPL	305
	THDEHIYWQPYALPL	306
	MLEKTYTTWTPG YWQPYALPL	307
	WSDPLTRDADLYWQPYALPL	308
15	SDAFTTQDSQAMYWQPYALPL	309
	GDDAAWRDSDLTYWQPYALPL	310
	AIIRQLYRWSEMYWQPYALPL	311
	ENTYSPNWADSMYWQPYALPL	312
	MNDQTSEVSTFPYWQPYALPL	313
20	SVGEDHNFWTSEYWQPYALPL	314
	QTPFTWEESNAYYWQPYALPL	315
	ENPFTWQESNAYYWQPYALPL	316
	VTPFTWEDSNVF YWQPYALPL	317
	QIPFTWEQSNAYYWQPYALPL	318
25	QAPLWQESAAYYWQPYALPL	319
	EPTFTWEESKAT YWQPYALPL	320
	TTTLTWEESNAYYWQPYALPL	321
	ESPLTWEESALYWQPYALPL	322
	ETPLTWEESNAYYWQPYALPL	323
30	EATFTWAESNAYYWQPYALPL	324
	EALFTWKESTAYYWQPYALPL	325
	STP-TWEESNAYYWQPYALPL	326
	ETPFTWEESNAYYWQPYALPL	327
	KAPFTWEESQAYYWQPYALPL	328
35	STSFTWEESNAYYWQPYALPL	329

	DSTFTWEESNAYYWQPYALPL	330
	YIPFTWEESNAYYWQPYALPL	331
	QTAFTWEESNAYYWQPYALPL	332
	ETLFTWEESNAT YWQPYALPL	333
5	VSSFTWEESNAYYWQPYALPL	334
	QPYALPL	335
	Py-1-NapPYQJYALPL	336
	TANVSSFEWTPG YWQPYALPL	337
	FEWTPGYWQPYALPL	338
10	FEWTPGYWQJYALPL	339
	FEWTPGYYQJYALPL	340
	ETPFTWEESNAYYWQPYALPL	341
	FTWEESNAYYWQJYALPL	342
	ADVLYWQPYAPVTLWV	343
15	GDVAEYWQPYALPLTSL	344
	SWTDYGYWQPYALPISGL	345
	FEWTPGYWQPYALPL	346
	FEWTPGYWQJYALPL	347
	FEWTPGWYQPYALPL	348
20	FEWTPGWYQJYALPL	349
	FEWTPGYYQPYALPL	350
	FEWTPGYYQJYALPL	351
	TANVSSFEWTPGYWQPYALPL	352
	SWTDYGYWQPYALPISGL	353
25	ETPFTWEESNAWAIQPYALPL	354
	ENTYSPNWADSMYWQPYALPL	355
	SVGEDHNFWTSEYWQPYALPL	356
	DGYDRWRQSGERYWQPYALPL	357
	FEWTPGYWQPYALPL	358
30	FEWTPGYWQPY	359
	FEWTPGYWQJY	360
	EWTPGYWQPY	361
	FEWTPGWYQJY	362
	AEWTPGYWQJY	363
35	FAWTPGYWQJY	364



	FEATPGYWQJY	365
	FEWAPGYWQJY	366
	FEWTAGYWQJY	367
	FEWTPAYWQJY	368
5	FEWTPGAWQJY	369
	FEWTPGYAQJY	370
	FEWTPGYWQJA	371
	FEWTGGYWQJY	372
	FEWTPGYWQJY	373
10	FEWTJGYWQJY	374
	FEVff PecGYWQJY	375
	FEWTPAibYWQJY	376
	FEWTPSarWYQJY	377
	FEWTSarGYWQJY	378
15	FEWTPNYWQJY	379
	FEWTPVYWQJY	380
	FEWTVPYWQJY	381
	AcFEWTPGVVYQJY	382
	AcFEVffPGYWQJY	383
20	INap-EVff PGYYQJY	384
	YEWTPGYYQJY	385
	FEWVPGYYQJY	386
	FEVff PGYYQJY	387
	FEVff PsYYQJY	388
25	FEWTPnYYQJY	389
	SHLY-Nap-QPYSVQM	390
	TLVY-Nap-LDPYSLQT	391
	RGDY-Nap-QPYSVQS	392
	NMVY-Nap-QPYSIQT	393
30	VYWQPYSVQ	394
	VY-Nap-QPYSVQ	395
	TFVYWQJYALPL	396
	FEWTPGYYQJ-Bpa	397
	XaaFEWTPGYYQJ-Bpa	398
35	FEWTPGY-Bpa-QJY	399

	AcFEWTPGY-Bpa-QJY	400
	FEWTPG-Bpa-YQJY	401
	AcFEWTPG-Bpa-YQJY	402
	AcFE-Bpa-TPGYYQJY	403
5	AcFE-Bpa-TPGYYQJY	404
	Bpa-EWTPGYYQJY	405
	AcBpa-EWTPGYYQJY	406
	VYWQPYSVQ	407
	RLVYWQPYSVQR	408
10	RLVY-Nap-QPYSVQR	409
	RLDYWQPYSVQR	410
	RLVWFQPYSVQR	411
	RLVYWQPYSIQR	412
	DNSSWYDSFLL	413
15	DNTAWYESFLA	414
	DNTAWYENFLL	415
	PAREDNTAWYDSFLIWC	416
	TSEYDNTTWYEKFLASQ	417
	SQIPDNTAWYQSFLHGH	418
20	SPFIDNTAWYENFLLTY	419
	EQIYDNTAWYDHFLLSY	420
	TPFIDNTAWYENFLLTY	421
	TYTYDNTAWYERFLMSY	422
	TMTQDNTAWYENFLLSY	423
25	TIDNTAWYANLVQTPQ	424
	TIDNTAWYERFLAQYPD	425
	HIDNTAWYENFLLTYTP	426
	SQDNTAWYENFLLSYKA	427
	QIDNTAWYERFLLQYNA	428
30	NQDNTAWYESFLLQYNT	429
	TIDNTAWYENFLLNHNL	430
	HYDNTAWYERFLQQGWH	431
	ETPFTWEESNAYYWQPYALPL	432
	YIPFTWEESNAYYWQPYALPL	433
35	DGYDRWRQSGERYWQPYALPL	434

	pY-INap-pY-QJYALPL	435
	TANVSSFEWTPGYWQPYALPL	436
	FEWTPGYWQJYALPL	437
	FEWTPGYWQPYALPLSD	438
5	FEWTPGYYQJYALPL	439
	FEWTPGYWQJY	440
	AcFEVVTPGYWQJY	441
	AcFEWTPGWYQJY	442
	AcFEWTPGYYQJY	443
10	AcFEWTPaYWQJY	444
	AcFEWTPaWYQJY	445
	AcFEWTPaYYQJY	446
	FEWTPGYYQJYALPL	447
	FEWTPGYWQJYALPL	448
15	FEWTPGWYQJYALPL	449
	TANVSSFEWTPGYWQPYALPL	450
	AcFEWTPGYWQJY	451
	AcFEWTPGWYQJY	452
	AcFEWTPGYYQJY	453
20	AcFEWTPAYWQJY	454
	AcFEWTPAWYQJY	455
	AcFEWTPAYYQJY	456

**Table 3-TPO-mimetic peptide sequences**

equence/structure	SEQ-ID-NO:
EGPTLRQWLAARA	457
IEGPTLRQWLAACA	458
IEGPTLREWLAARA	459
IEGPTLRQWLAARA-A- IEGPTLRQWLAARA	460
IEGPTLRQWLAACA-A- IEGPTLRQWLAACA	461
IEGPTLRQCLAARA-A- IEGPTLRQCLAARA	462

equence/structure	SEQ-ID-NO:
IEGPTLRQWLAARA-A-K(BrAc)-A- IEGPTLRQWLAARA	463
IEGPTLRQWLAARA-A-K(PEG)-A- IEGPTLRQWLAARA	464
IEGPTLRQCLAARA-A- IEGPTLRQWLAARA	465
IEGPTLRQCLAARA-Δ- IEGPTLRQCLAARA	466
IEGPTLRQWLAARA-A- IEGPTLRQULA/AtIA	467
VRDQIXXXL	468
TLREWL	469
GRVRDQVAGW	470
GRVKDQIAQL	471
GVRDQVSWAL	472
ESVREQVMKY	473
SVRSQISASL	474
GVRETVYRHM	475
GVREVIVMHML	476
GRVRDQIWAAL	477
AGVRDQILIWL	478
GRVRDQIMLSL	479
GRVRDQI(X) <sub>3</sub> L	480
CTLRQWLQGC	481
CTLQEFLEGC	482
CTRTEWLHGC	483
CTLREWLHGGFC	484
CTLREWVFAGLC	485
CTLRQWLILLGMC	486
CTLAEFSLASGVEQC	487
CSLQEFLSHGGYVC	488
CTLREFLDPTTAVC	489
CTLKEWLVSHEVWC	490

sequence/structure	SEQ-ID-NO:
CTLREWL(X) <sub>2-6</sub> C	491-495
REGPTLRQWM	496
EGPTLRQWLA	497
ERGPFWAKAC	498
REGPRCVMWM	499
CGTEGPTLSTWLDC	500
CEQDGPTLLEWLKC	501
CELVGPSLMSWLTC	502
CLTGPFVTQWLYEC	503
CRAGPTLLEWLTLC	504
CADGPTLREWISFC	505
C(X) <sub>1-2</sub> EGPTLREWL(X) <sub>1-2</sub> C	506-510
GGCTLREWLHGGFCGG	511
GGCADGPTLREWISFCGG	512
GNADGPTLRQWLEGRPKN	513
LAIEGPTLRQWLHGNGRDT	514
HGRVGPTLREWKTQVATKK	515
TIKGPTLRQWLKSREHTS	516
ISDGPTLKEWLSVTRGAS	517
SIEGPTLREWLTSRTPHS	518

**Table 4-G-CSF-mimetic peptide sequences**

5	Sequence/structure	SEQ ID NO:
	EEDCK	519
	EED $\alpha$ K	520
	pGluED $\alpha$ K	521
	PicSD $\alpha$ K	522
10	EEDCK- $\Delta$ -EEDCK	523
	EEDXK- $\Delta$ -EEDXK	524

**Table 5-TNF-antagonist peptide sequences**

	Sequence/structure	SEQID NO:
	YCFTASENH CY	525
	YCFTNSENH CY	526
5	YCFTRSENH CY	527
	FCASENH CY	528
	YCASENH CY	529
	FCNSENH CY	530
	FCNSENRCY	531
10	FCNSVENRCY	532
	YCSQSVSND CF	533
	FCVSNDRCY	534
	YCRKELGQVCY	535
	YCKEPGQCY	536
15	YCRKEMGCY	537
	FCRKEMGCY	538
	YCWSQNLCY	539
	YCELSQYLCY	540
	YCWSQNYCY	541
20	YCWSQYLCY	542
	DFLPHYKNTSLGHRP	543

**Table 6-Integrin-binding peptide sequences**

	Sequence/structure	SEQID NO:
25	RX <sub>1</sub> ETX <sub>2</sub> WX <sub>3</sub>	544
	RX <sub>1</sub> ETX <sub>2</sub> WX <sub>3</sub>	545
	RGDGX	546
	CRGDGXC	547
30	CX <sub>1</sub> X <sub>2</sub> RLDX <sub>3</sub> X <sub>4</sub> C	548
	CARRLDAPC	549
	CPSRLDSPC	550
	X <sub>1</sub> X <sub>2</sub> X <sub>3</sub> RGDX <sub>4</sub> X <sub>5</sub> X <sub>6</sub>	551
	CX <sub>2</sub> CRGDCX <sub>5</sub> C	552

	WO 03/084477	PCT/US03/09139
	CDCRGDCFC	553
	CDCRGDCLC	554
	CLCRGDCIC	555
	X <sub>1</sub> X <sub>2</sub> DDX <sub>4</sub> X <sub>5</sub> X <sub>7</sub> X <sub>8</sub>	556
5	X <sub>1</sub> X <sub>2</sub> X <sub>3</sub> DDX <sub>4</sub> X <sub>5</sub> X <sub>6</sub> X <sub>7</sub> X <sub>8</sub>	557
	CWDDGWL	558
	CWDDLWWLC	559
	CWDDGLMC	560
	CWDDGWMC	561
10	CSWDDGWLC	562
	CPDDLWWLC	563
	NGR	NR
	GSL	NR
	RGD	NR
15	CGRECPRLCQSSC	564
	CNGRCVSGCAGRC	565
	CLSGSLSC	566
	RGD	NR
	NGR	NR
20	GSL	NR
	NGRAHA	567
	CNGRC	568
	CDCRGDCFC	569
	CGSLVRC	570
25	DLXXL	571
	RTDLDSLRTYTL	572
	RTDLDSLRTY	573
	RTDLDSLRT	574
	RTDLDSLRL	575
30	GDLDLLKLRLTL	576
	GDLHSLRQLLSR	577
	RDDLHMLRLQLW	578
	SSDLHALKKRYG	579
	RGDLKQLSELTW	580
35	RGDLAALSAPPV	581

**Table 7-Selectin antagonist peptide sequences**

	Sequence/structure	SEQ ID NO:
5	DITWDQLWDLMK	582
	DITWDELWKIMN	583
	DYTWFEWDMMQ	584
	QITWAQLWNMMK	585
	DMTWHDLWTLMS	586
10	DYSWHDLWEMMS	587
	EITWDQLWEVMN	588
	HVSWEQLWDIMN	589
	HITWDQLWRIMT	590
	RNMSWLELWEHMK	591
15	AEWTWDQLWHVMNPAESQ	592
	HRAEWLALWEQMSP	593
	KKEDWLALWRIMSV	594
	ITWDQLWDLMK	595
	DITWDQLWDLMK	596
20	DITWDQLWDLMK	597
	DITWDQLWDLMK	598
	CQNRYTDLVAIQNKNE	599
	AENWADNEPNNKRNNED	600
	RKNNKTWTWVGTTKALTNE	601
25	KKALTNEAENWAD	602
	CQXRYTDLVAIQNKXE	603
	RKXNXXWTWVGTXKXLTEE	604
	AENWADGEPNNKXNXED	605
	CXXXYTXXLVAIQNKXE	606
30	RKXXXXWXWVGTXKXLTXE	607
	AXNWXXXEPNNXXXED	608
	XKXKTXEAXNWXX	609

**Table 8-Antipathogenic peptide sequences**

35	Sequence/structure	SEQ ID NO:
----	--------------------	------------



	GFFALIPKIISSPLFKTLLSAVGSALSSSGGQQ	610
	GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE	611
	GFFALIPKIISSPLFKTLLSAV	612
5	GFFALIPKIISSPLFKTLLSAV	613
	KGFFALIPKIISSPLFKTLLSAV	614
	KKGFFALIPKIISSPLFKTLLSAV	615
	KKGFFALIPKIISSPLFKTLLSAV	616
	GFFALIPKIISS	617
10	GIGAVLKVLTTGLPALISWIKRKRQQ	618
	GIGAVLKVLTTGLPALISWIKRKRQQ	619
	GIGAVLKVLTTGLPALISWIKRKRQQ	620
	GIGAVLKVLTTGLPALISWIKR	621
	AVLKVLTTGLPALISWIKR	622
15	KLLLLLKLKK	623
	KLLKLKK	624
	KLLKLKK	625
	KKLLKLKK	626
	KLLKLKK	627
20	KLLKLKK	628
	KLLK	629
	KLLKLK	630
	KLLKLKK	631
	KLLKLKK	632
25	KLLKLKK	633
	KAAAKAAAKAAK	634
	KVVVKVVVKVK	635
	KVVVKVKVKVK	636
	KVVVKVKVKVK	637
30	KVVVKVKVKVK	638
	KLILKL	639
	KVLHLL	640
	LKLRL	641
	KPLHLL	642
35	KLILKLVR	643

	KVFHLLHL	644
	HKFRILKL	645
	KPFHILHL	646
	KHKKIKIKIK	647
5	KIKIKIKIKIK	648
	KIKIKIKIKIK	649
	KIPIKIKIKIPK	650
	KIPIKIKIKIVK	651
	RIIRIRIRIR	652
10	RIIRIRIRIR	653
	RIIRIRIRIR	654
	RIVIRIRIRLIR	655
	RIIVRIRLRIR	656
	RIGIRLRVRIR	657
15	KIVIRIRIRLIR	658
	RIAVKWRLRFIK	659
	KIGWKLVRIR	660
	KKIGWLIIRVRR	661
	RIVIRIRIRLIR	662
20	RIIVRIRLRIRVR	663
	RIGIRLRVRIRRV	664
	KIVIRIRARLIRIR	665
	RIIVKIRLRIRKIRL	666
	KIGIKARVRIRVKIR	667
25	RIIVHIRLRIRHIRL	668
	HIGIKAHVRIRVHIR	669
	RIYVKIHLRYIKKIRL	670
	KIGHKARVHIRYKIR	671
	RIYVKPHPRYIKKIRL	672
30	KPGHKARPHIRYKIR	673
	KIVIRIRIRLIRIRKIRIV	674
	RIIVKIRLRIRKIRLIKK	675
	KIGWKLVRIRVKIGRLR	676
	KI.VIRIRIRLIRIRKIRIVKVKIR	677
35	RFAVKIRLRIRKIRLIKKIRKRVIR	678

	KAGWKLRVRIIRVKIGRLRKIGWKKRVRK	679
	RIYVKPHPRYIKKIRL	680
	KPGHKARPHIIRYKII	681
	KIVIRIRIRLIRIRIRKIV	682
5	RIIVKIRLRIKKIRLIKK	683
	RIYVSKISYIKKIRL	684
	KIVIFTRIRLTSIRIRSIV	685
	KPIHKARPTIIRYKMI	686
	cyclicCKGFFALIPKIISSPLFKTLLSAVC	687
10	CKKGFFALIPKIISSPLFKTLLSAVC	688
	CKKKGFFALIPKIISSPLFKTLLSAVC	689
	CyclicCRIVIRIRIRLIRIRC	690
	CyclicCKPGHKARPHIIRYKIIC	691
	CyclicCRFAVKIRLRIKKIRLIKKIRKRVIKC	692
15	KLLLKLLL KLLKC	693
	KLLLKLLLKLLK	694
	KLLLKLLKLLKC	695
	KLLLKLLLKLLK	696

# 20 **Table 9-VIP-mimetic peptide sequences**

	Sequence/structure	SEQ ID NO:
	HSDAVFYDNYTR LRKQMAVKKYLN SILN	697
	Me HSDAVFYDNYTR LRKQMAVKKYLN SILN	698
	X <sub>1</sub> X <sub>1</sub> ' X <sub>1</sub> " X <sub>2</sub>	699
25	X <sub>3</sub> SX <sub>4</sub> LN	700
	KKYL	701
	NSILN	702
	KKYL	703
	KKYA	704
30	AVKKYL	705
	NSILN	706
	KKYV	707
	SILauN	708
	KKYLNle	709
35	NSYLN	710

	NSIYN	711
	KKYLPPNSILN	712
	LauKKYL	713
	CapKKYL	714
5	KYL	NR
	KKYNIe	715
	VKKYL	716
	LNSILN	717
	YLNSILN	718
10	KKYLN	719
	KKYLNS	720
	KKYLNSI	721
	KKYLNSIL	722
	KKYL	723
15	KKYDA	724
	AVKKYL	725
	NSILN	726
	KKYV	727
	SILauN	728
20	NSYLN	729
	NSIYN	730
	KKYLNie	731
	KKYLPPNSILN	732
	KKYL	733
25	KKYDA	734
	AVKKYL	735
	NSILN	736
	IKKYV	737
	SILauN	738
30	LauKKYL	739
	CapKKYL	740
	KYL	NR
	KYL	NR
	KKYNIe	741
35	VKKYL	742

	LNSILN	743
	YLNSILN	744
	KKYLNle	745
	KKYLN	746
5	KKYLNS	747
	KKYLNSI	748
	KKYLNSIL	749
	KKKYLD	750
	cyclicCKKYLC	751
10	CKKYLK	752
	KKYA	753
	WWTDTGLW	754
	WWTDDGLW	755
	WWDTRGLWVWTI	756
15	FWGNDGIWLESG	757
	DWDQFGLWRGAA	758
	RWDDNGLWVVVL	759
	SGMWSHYGIWMG	760
	GGRWDQAGLWVA	761
20	KLWSEQGIWMGE	762
	CWSMHGLWLC	763
	GCWDNTGIWVPC	764
	DWDTRGLWVY	765
	SLWDENGAWI	766
25	KWDDRGLWMH	767
	QAWNERGLWT	768
	QWDTRGLWVA	769
	WNVHGIWQE	770
	SWDTRGLWVE	771
30	DWDTRGLWVA	772
	SWGRDGLWIE	773
	EWTDNGLWAL	774
	SWDEKGLWSA	775
	SWDSSGLWMD	776

35

**Table 10-Mdm/hdm antagonist peptide sequences**

	Sequence/structure	SEQID NO:
	TFSDLW	777
	QETFSDLWKLLP	778
5	QPTFSDLWKLLP	779
	QETFSDYWKLLP	780
	QPTFSDYWKLLP	781
	MPRFMDYWEGLN	782
	VQNFIDYWTQQF	783
10	TGPAFTHYWATF	784
	IDRAPTFRDHWFALV	785
	PRPALVFADYWETLY	786
	PAFSRFWSDL SAGAH	787
	PAFSRFWSKLSAGAH	788
15	PXFXDYWXXL	789
	QETFSDLWKLLP	790
	QPTFSDLWKLLP	791
	QETFSDYWKLLP	792
	QPTFSDYWKLLP	793
20		

**Table 11-Calmodulin antagonist peptide sequences**

	Sequence/structure	SEQ ID NO:
	SCVKWGGKEFCGS	794
25	SCWKYWGKECGS	795
	SCYEWGKL RWCGS	796
	SCLR WGKWSNCGS	797
	SCWRWGKYQICGS	798
	SCVSWGALKLCGS	799
30	SCIRWGQNTFCGS	800
	SCWQWGNLKICGS	801
	SCVRWGQLSICGS	802
	LKKFNARRKLKGAILTTMLAK	803
	RRWKKNFI AVSAANRFKK	804
35	RKWQKTGHAVRAIGRLSS	805

	INLKALAALAKKIL	806
	KIWSILAPLGTTLVKLVA	807
	LKKLLKLLKKLLKL	808
	LKWKKLLKLLKKLLKKLL	809
5	AEWPSLTEIKTLSHFSV	810
	AEWSPTRVISTTYFGS	811
	AELAHWPPVKTVLRSFT	812
	AEGSWLQLLNLMKQMNN	813
	AEWPSLTEIK	814

10

**Table 12-Mast cell antagonists/Mast cell protease inhibitor peptide sequences**

	Sequence/structure	SEQ ID NO:
	SGSGVLKRPLPILPVTR	815
15	RWLSSRPLPPLPLPPRT	816
	GSGSYDTLALPSLPLHPMSS	817
	GSGSYDTRALPSLPLHPMSS	818
	GSGSSGVTMYPKLPPHWSMA	819
	GSGSSGVARMYPKLPPHWSMA	820
20	GSGSSSMRMVPTIPGSAKHG	821
	RNR	NR
	QT	NR
	RQK	NR
	NRQ	NR
25	RQK	NR
	RNRQKT	822
	RNRQ	823
	RNRQK	824
	NRQKT	825
30	RQKT	826

**Table 13-SH3 antagonist peptide sequences**

	Sequence/structure	SEQ ID NO:
35	RPLPPLP	827

	RELPPLP	828
	SPLPPLP	829
	GPLPPLP	830
	RPLPIP	831
5	RPLPIP	832
	RRLPPTP	834
	RQLPPTP	835
	RPLPSRP	836
	RPLPTRP	837
10	SRLPPLP	838
	RALPSPP	839
	RRLPRTT	840
	RPVPPIT	841
	ILAPPVP	842
15	RPLPMLP	843
	RPLPILP	844
	RPLPSLP	845
	RPLPSLP	846
	RPLPMIP	847
20	RPLPLIP	848
	RPLPPTP	849
	RSLPPLP	850
	RPQPPPP	851
	RQLPIP	852
25	XXXRPLPPLPXP	853
	XXXRPLPPIPXX	854
	XXXRPLPPLPXX	855
	RXXRPLPPLPXP	856
	RXXRPLPPLPPP	857
30	PPPYPPPPIPXX	858
	PPPYPPPPVPXX	859
	LXXRPLPXTT	860
	ΨXXRPLPXLP	861
	PPX⊗XPPPΨP	862



	+PPΨPXKPXWL	863
	RPXΨPΨR+SXP	864
	PPVPPRPXXTL	865
	ΨPΨLPΨK	866
5	+ΘDXPLPXL	867

**Table 14-Somatostatin or cortistatin mimetic peptide sequences**

	Sequence/structure	SEQID NO:
10	X <sup>1</sup> X <sup>2</sup> -Asn-Phe-Phe-Trp-Lys-Thr-Phe-X <sup>3</sup> -Ser-X <sup>4</sup>	868
	Asp Arg Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys	869
	Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys	870
	Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys	871
	Asp Arg Met Pro_Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	872
15	Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	873
	Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	874
	Asp Arg Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	875
	Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys	876
	Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys	877
20	Asp Arg Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	878
	Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	879
	Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys	880
	Asp Arg Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	881
	Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	882
25	Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	883
	Asp Arg Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	884
	Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	885
	Cys Arg Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	886
	Asp Arg Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	887
30	Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	889
	Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys Lys	890
	Asp Arg Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	891
	Met Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	892
	Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys	893

**Table 15-UKR antagonist peptide sequences**

	Sequence/structure	SEQID NO:
5	AEPMPHSLNFSQYLWYT	894
	AEHTYSSLWDTYSPLAF	895
	AELDLWMRHYPLSFSNR	896
	AESSLWTRYAWPSMPSY	897
	AEWHPGLSFGSYLWSKT	898
10	AEPALLNWSFFFNPLH	899
	AEWSFYNLHLPEPQTIF	900
	AEPLDLWSLYSLPPLAM	901
	AEPTLWQLYQFPLRLSG	902
	AEISFSELMWLRSTPAF	903
15	AELSEADLWTTWFGMGS	904
	AESSLWRIFSPSALMMS	905
	AESLPTLTSILWGKESV	906
	AETLFMDLWHDKHILLT	907
	AEILNFPLWHEPLWSTE	908
20	AESQTGTLNTLFWNTLR	909
	AEPVYQYELDSYLRSY	910
	AELDLSTFYDIQYLLRT	911
	AEFFKLGPNGYVYLHSA	912
	FKLXXXGYVYL	913
25	AESTYHHLSLGYMYTLN	914
	YHXLXXGYMYT	915

**Table 16-Macrophage and/or T-cell inhibiting peptide sequences**

	Sequence/structure	SEQID NO:
	Xaa-Yaa-Arg	NR
5	Arg-Yaa-Xaa	NR
	Xaa-Arg-Yaa	NR
	Yaa-Arg-Xaa	NR
	Ala-Arg	NR
	Arg-Arg	NR
10	Asn-Arg	NR
	Asp-Arg	NR
	Cys-Arg	NR
	Gln-Arg	NR
	Glu-Arg	NR
15	Gly-Arg	NR
	His-Arg	NR
	Ile-Arg	NR
	Leu-Arg	NR
	Lys-Arg	NR
20	Met-Arg	NR
	Phe-Arg	NR
	Ser-Arg	NR
	Thr-Arg	NR
	Trp-Arg	NR
25	Tyr-Arg	NR
	Val-Arg	NR
	Ala-Glu-Arg	NR
	Arg-Glu-Arg	NR
	Asn-Glu-Arg	NR
30	Asp-Glu-Arg	NR
	Cys-Glu-Arg	NR
	Gln-Glu-Arg	NR
	Glu-Glu-Arg	NR
	Gly-Glu-Arg	NR
35	His-Glu-Arg	NR

	Ile-Glu-Arg	NR
	Leu-Glu-Arg	NR
	Lys-Glu-Arg	NR
	Met-Glu-Arg	NR
5	Phe-Glu-Arg	NR
	Pro-Glu-Arg	NR
	Ser-Glu-Arg	NR
	Thr-Glu-Arg	NR
	Trp-Glu-Arg	NR
10	Tyr-Glu-Arg	NR
	Val-Glu-Arg	NR
	Arg-Ala	NR
	Arg-Asp	NR
	Arg-Cys	NR
15	Arg-Gln	NR
	Arg-Glu	NR
	Arg-Gly	NR
	Arg-His	NR
	Arg-Ile	NR
20	Arg-Leu	NR
	Arg-Lys	NR
	Arg-Met	NR
	Arg-Phe	NR
	Arg-Pro	NR
25	Arg-Ser	NR
	Arg-Thr	NR
	Arg-Trp	NR
	Arg-Tyr	NR
	Arg-Val	NR
30	Arg-Glu-Ala	NR
	Arg-Glu-Asn	NR
	Arg-Glu-Asp	NR
	Arg-Glu-Cys	NR
	Arg-Glu-Gln	NR
35	Arg-Glu-Glu	NR

	Arg-Glu-Gly	NR
	Arg-Glu-His	NR
	Arg-Glu-Ile	NR
	Arg-Glu-Leu	NR
5	Arg-Glu-Lys	NR
	Arg-Glu-Met	NR
	Arg-Glu-Phe	NR
	Arg-Glu-Pro	NR
	Arg-Glu-Ser	NR
10	Arg-Glu-Thr	NR
	Arg-Glu-Trp	NR
	Arg-Glu-Tyr	NR
	Arg-Glu-Val	NR
	Ala-Arg-Glu	NR
15	Arg-Arg-Glu	NR
	Asn-Arg-Glu	NR
	Asp-Arg-Glu	NR
	Cys-Arg-Glu	NR
	Gln-Arg-Glu	NR
20	Glu-Arg-Glu	NR
	Gly-Arg-Glu	NR
	His-Arg-Glu	NR
	Ile-Arg-Glu	NR
	Leu-Arg-Glu	NR
25	Lys-Arg-Glu	NR
	Met-Arg-Glu	NR
	Phe-Arg-Glu	NR
	Pro-Arg-Glu	NR
	Ser-Arg-Glu	NR
30	Thr-Arg-Glu	NR
	Trp-Arg-Glu	NR
	Tyr-Arg-Glu	NR
	Val-Arg-Glu	NR
	Glu-Arg-Ala	NR
35	Glu-Arg-Arg	NR

	Glu-Arg-Asn	NR
	Glu-Arg-Asp	NR
	Glu-Arg-Cys	NR
	Glu-Arg-Gln	NR
5	Glu-Arg-Gly	NR
	Glu-Arg-His	NR
	Glu-Arg-Ile	NR
	Glu-Arg-Leu	NR
	Glu-Arg-Lys	NR
10	Glu-Arg-Met	NR
	Glu-Arg-Phe	NR
	Glu-Arg-Pro	NR
	Glu-Arg-Ser	NR
	Glu-Arg-Thr	NR
15	Glu-Arg-Trp	NR
	Glu-Arg-Tyr	NR
	Glu-Arg-Val	NR

**Table 17-Additional Exemplary Pharmacologically Active Peptides**

20	Sequence/Structure	SEQID NO:	Activity
	VEPNCDIHVMWEWECFERL	916	VEGF-antagonist
	GERWCDFDGPLTWVCGEES	917	VEGF-antagonist
25	RGWVEICVADDNGMCVTEAQ	918	VEGF-antagonist
	GWDECDVARMWEWECFAGV	919	VEGF- antagonist
	GERWCDFDGPRAWVCGWEI	920	VEGF- antagonist
	EELWCDFDGPRAWVCGYVK	921	VEGF- antagonist
	RGWVEICAADDYGRCLTEAQ	922	VEGF- antagonist
30	RGWVEICESDVWGRCL	923	VEGF- antagonist
	RGWVEICESDVWGRCL	924	VEGF- antagonist
	GGNECDIARMWEWECFERL	925	VEGF- antagonist
	RGWVEICAADDYGRCL	926	VEGF-antagonist
	CTTHWGFTLC	927	MMP inhibitor
35	CLRSGXGC	928	MMP inhibitor

	CXXHWGFXXC	929	MMP inhibitor
	CXPXC	930	MMP inhibitor
	CRRHWGFEC	931	MMP inhibitor
	STTHWGF TLS	932	MMP inhibitor
5	CSLHWGFWWC	933	CTLA4-mimetic
	GFVCSGIFAVGVGRC	934	CTLA4-mimetic
	APGVRLGCAVLGRYC	935	CTLA4-mimetic
	LLGRMK	936	Antiviral (HBV)
	ICVVQDWGHHRCTAGHMANLTSHASAI	937	C3b antagonist
10	ICVVQDWGHHRCT	938	C3b antagonist
	CVVQDWGHHAC	939	C3b antagonist
	STGGFDDVYDWARGVSSALTTTLVATR	940	Vinculin-binding
	STGGFDDVYDWARRVSSALTTTLVATR	941	Vinculin-binding
	SRGVNFSEWLYDMSAAMKEASNVFPSRRSR	942	Vinculin-binding
15	SSQNWDMEAGVEDLTAAMLGLLSTIHSSSR	943	Vinculin-binding
	SSPSLYTQFLVNYESAATRIQDLLIASRPSR	944	Vinculin-binding
	SUGMIDILLGAILQRAADATRTSIPIPSLQNSIR	945	Vinculin-binding
	DVYTKKELIECARRVSEK	946	Vinculin-binding
	EKGSYYPGSGIAQFHIDYNNVS	947	C4BP-binding
20	SGIAQFHIDYNNVSSAEGWHVN	948	C4BP-binding
	LVTVEKGSYYPGSGIAQFHIDYNNVSSAEGWHVN	949	4BP-binding
	SGIAQFHIDYNNVS	950	C4BP-binding
	LLGRMK	951	anti-HBV
	ALLGRMKG	952	anti-HBV
25	LDPAFIR	953	anti-HBV
	CXXRGDC	954	Inhibition of platelet aggregation
	RPLPPLP	955	Src antagonist
	PPVPPR	956	Src antagonist
	XFXDXWXXLXX	957	Anti-cancer
30	KACRRLFGPVDSEQLSRDCD	958	pl 6-mimetic
	RERWNFDVFTETPLEGDFAW	959	pl 6-mimetic
	KRRQTSMTDFYHSKRRLIFS	960	pl 6-mimetic
	TSM TDFYHSKRRLIFSKRKP	961	pl 6-mimetic
	RRLIF	962	p16-mimetic
35	KRRQTSATDFYHSKRRLIFSRQIKIWFQNRRMKWKK	963	p16-mimetic

	KRRLIFSKRQIKIWFQNRRMKWKK	964	pl 6-mimetic
	Asn Gin Gly Arg His Phe Cys Gly Gly Ala Leu Ile His Ala Arq Phe Val Met Thr Ala Ala Ser		
	Cys Phe Gln	965	CAP37 mimetic/LPs bindin
	Arg His Phe Cys Gly Gly Ala Leu Ile His Ala Arg Phe Val Met Thr Ala Ala Ser Cys	499	
5	CAP37 mimetic/LPS binding		
	Gly Thr Arg Cys Gin Val Ala Gly Trp Gly Ser Gln Arg Ser Gly Gly Arg Leu Ser Arg Phe Pro		
	Arg Phe Val Asn Val	966	CAP37 mimetic/LPS binding
	WHWRHRIPLQLAAGR	967	carbohydrate (GID1 alpha) mimetic
	LKTPRV	968	I32GPI Ab binding
10	NTLKTPRV	969	I32GPI Ab binding
	NTLKTPRVGGC	970	02GPI Ab binding
	KDKATF	971	02GPI Ab binding
	KDKATFGCHD	972	P2GPI Ab binding
	KDKATFGCHDGC	973	02GPI Ab binding
15	TLRVYK	974	02GPI Ab binding
	ATLRVYKG	975	02GPI Ab binding
	CATLRVYKGG	976	132GPI Ab binding
	INLKALAALAKKIL	977	Membrane transporting
	GWT	NR	Membrane transporting
20	GWTLNSAGYLLG	978	Membrane transporting
	GWTLNSAGYLLGKINLKALAALAKKIL	979	Membrane transporting

The present invention is also particularly useful with peptides having activity in treatment of: a VEGF related condition, e.g., but not limited to, cancer, wherein the peptide is a

25 VEGF-mimetic or a VEGF receptor antagonist, a HER2 agonist or antagonist, a CD20 antagonist and the like; asthma, wherein the protein of interest is a CKR3 antagonist, an IL-5 receptor antagonist, and the like; thrombosis, wherein the protein of interest is a GPIIb antagonist, a GPIIIa. antagonist, and the like; autoimmune diseases and other conditions involving immune modulation, wherein the protein of interest is an IL-2 receptor antagonist, a

30 CD40 agonist or antagonist, a CD40L agonist or antagonist, a thymopoietin mimetic and the like.

For example, EPO biological activities are well known in the art. See, e.g., Anagnostou A et al Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. Proceedings of the National Academy of Science (USA) 87: 5978-82 (1990); Fandrey J and Jelkman WE Interleukin 1 and

35 tumor necrosis factor-alpha inhibit erythropoietin production in vitro. Annals of the New York Academy



of Science 628: 250-5 (1991); Geissler K et al Recombinant human erythropoietin: A multipotential hemopoietic growth factor in vivo and in vitro. *Contrib. Nephrol.* 87: 1-10 (1990); Gregory CJ Erythropoietin sensitivity as a differentiation marker in the hemopoietic system. *Studies of three erythropoietic colony responses in culture.* *Journal of Cellular Physiology* 89: 289-301 (1976); Jelkman W et al Monokines inhibiting erythropoietin production in human hepatoma cultures and in isolated perfused rat kidneys. *Life Sci.* 50: 301-8 (1992); Kimata H et al Human recombinant erythropoietin directly stimulates B cell immunoglobulin production and proliferation in serum-free medium. *Clinical and Experimental Immunology* 85: 151-6 (1991); Kimata H et al Erythropoietin enhances immunoglobulin production and proliferation by human plasma cells in a serum-free medium. *Clin. Immunology Immunopathol.* 59: 495-501 (1991); Kimata H et al Effect of recombinant human erythropoietin on human IgE production in vitro *Clinical and Experimental Immunology* 83: 483-7 (1991); Koury MJ and Bondurant MC Erythropoietin retards DNA breakdown and prevents programmed cell death in erythroid progenitor cells. *Science* 248: 378-81 (1990); Lim VS et al Effect of recombinant human erythropoietin on renal function in humans. *Kidney International* 37: 131-6 (1990); Mitjavila MT et al Autocrine stimulation by erythropoietin and autonomous growth of human erythroid leukemic cells in vitro. *Journal of Clinical Investigation* 88: 789-97 (1991); Andre M et al Performance of an immunoradiometric assay of erythropoietin and results for specimens from anemic and polycythemic patients. *Clinical Chemistry* 38: 758-63 (1992); Hankins WD et al Erythropoietin-dependent and erythropoietin-producing cell lines. Implications for research and for leukemia therapy. *Annals of the New York Academy of Science* 554: 21-8 (1989); Kendall RGT et al Storage and preparation of samples for erythropoietin radioimmunoassay. *Clin. Lab. Haematology* 13: 189-96 (1991); Krumvieh D et al Comparison of relevant biological assays for the determination of biological active erythropoietin. *Dev. Biol. Stand.* 69: 15-22 (1988); Ma DD et al Assessment of an EIA for measuring human serum erythropoietin as compared with RIA and an in-vitro bioassay. *British Journal of Haematology* 80: 431-6 (1992); Noe G et al A sensitive sandwich ELISA for measuring erythropoietin in human serum *British Journal of Haematology* 80: 285-92 (1992); Pauly JU et al Highly specific and highly sensitive enzyme immunoassays for antibodies to human interleukin 3 (IL3) and human erythropoietin (EPO) in serum. *Behring Institut Mitteilungen* 90: 112-25 (1991); Sakata S and Enoki Y Improved microbioassay for plasma erythropoietin based on CFU-E colony formation. *Ann. Hematology* 64: 224-30 (1992); Sanengen T et al Immunoreactive erythropoietin and erythropoiesis stimulating factor(s) in plasma from hypertransfused neonatal and adult mice. *Studies with a radioimmunoassay and a cell culture assay for erythropoietin.* *Acta Physiol. Scand.* 135: 11-6 (1989); Widness JA et al A sensitive and specific erythropoietin immunoprecipitation assay: application to pharmacokinetic studies. *Journal of Lab. Clin. Med.* 119: 285-94 (1992); for further information see also individual cell lines used in individual bioassays. Each of the above references are entirely incorporated herein by reference. EPO can be assayed by employing cell lines such as HCD57 , NFS-60 , TF-1 and

UT-7 , which respond to the factor . EPO activity can be assessed also in a Colony formation assay by determining the number of CFU-E from bone marrow cells. An alternative and entirely different detection method is RT-PCR quantitation of cytokines.

A CDR mimetibody, or specified portion or variant thereof, that partially or preferably substantially provides at least one biological activity of at least one protein or fragment, can bind the protein or fragment ligand and thereby provide at least one activity that is otherwise mediated through the binding of protein to at least one protein ligand or receptor or through other protein-dependent or mediated mechanisms. As used herein, the term "CDR mimetibody activity" refers to a CDR mimetibody that can modulate or cause at least one protein-dependent activity by about 20-10,000%, preferably by at least about 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 % or more depending on the assay.

The capacity of a CDR mimetibody or specified portion or variant to provide at least one protein-dependent activity is preferably assessed by at least one suitable protein biological assay, as described herein and/or as known in the art. A human CDR mimetibody or specified portion or variant of the invention can be similar to any class (IgG, IgA, IgM, etc.) or isotype and can comprise at least a portion of a kappa or lambda light chain, wherein at least one of the LBRs is replaced by at least one LBR as described herein. In one embodiment, the human CDR mimetibody or specified portion or variant comprises an IgG heavy chain or defined fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. In another embodiment, the human protein human CDR mimetibody or specified portion or variant thereof comprises an IgG1 heavy chain and a IgG1 light chain.

At least one CDR mimetibody or specified portion or variant of the invention binds at least one specified ligand specific to at least one protein, subunit, fragment, portion or any combination thereof. The at least one LBR of at least one CDR mimetibody, specified portion or variant of the present invention can optionally bind at least one specified ligand epitope of the ligand. The binding epitope can comprise any combination of at least one amino acid sequence of at least 1-3 amino acids to the entire specified portion of contiguous amino acids of the sequences selected from the group consisting of a protein ligand, such as a receptor or portion thereof.

Generally, the CDR mimetibody or ligand-binding fragment of the present invention can comprise a ligand binding region (LBR) (e.g., LBR1, LBR2 and LBR3) or variant provided in at least one heavy chain variable region and at least one ligand binding region (LBR1, LBR2 and LBR3) or variant provided in at least one light chain variable region. As a non-limiting example, the CDR mimetibody or ligand-binding portion or variant can comprise at least one of the heavy chain LBR3, and/or a light chain LBR3. In a particular embodiment, the CDR mimetibody or ligand-binding

fragment can have an ligand-binding region that comprises at least a portion of at least one heavy chain LBR (i.e., LBR1, LBR2 and/or LBR3) having the amino acid sequence of the corresponding LBRs 1, 2 and/or 3). In another particular embodiment, the CDR mimetibody or ligand-binding portion or variant can have an ligand-binding region that comprises at least a portion of at least one light chain LBR (i.e., LBR1, LBR2 and/or LBR3) having the amino acid sequence of the corresponding LBRs 1, 2 and/or 3 (e.g., SEQID NOS: 10, 11, and/or 12). Such mimetibodies can be prepared by joining together the various portions (e.g., LBRs, framework) of the CDR mimetibody using known techniques, by preparing and expressing at least one (i.e., one or more) nucleic acid molecules that encode the CDR mimetibody, using known techniques of recombinant DNA technology or by using any other suitable method, such as chemical synthesis.

The CDR mimetibody can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. Mimetibodies that bind to human protein ligands or receptors and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such as phage display (Katsube, Y., *et al.*, *Int J Mol. Med*, 1(5):863-868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. The CDR mimetibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

The invention also relates to mimetibodies, ligand-binding fragments, immunoglobulin chains and LBRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such mimetibodies or ligand-binding fragments and mimetibodies comprising such chains or LBRs can bind human protein ligands with high affinity (e.g.,  $K_D$  less than or equal to about  $10^{-9}$  M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g, charge, structure, polarity, hydrophobicity/ hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

#### Amino Acid Codes

The amino acids that make up mimetibodies or specified portions or variants of the present invention are often abbreviated. The amino acid designations can be indicated by designating the

amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994):

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
A	Ala	Alanine	GCA, GCC, GCG, GCU
C	Cys	Cysteine	UGC, UGU
D	Asp	Aspartic acid	GAC, GAU
E	Glu	Glutamic acid	GAA, GAG
F	Phe	Phenylalanine	UUC, UUU
G	Gly	Glycine	GGA, GGC, GGG, GGU
H	His	Histidine	CAC, CAU
I	Ile	Isoleucine	AUA, AUC, AUU
K	Lys	Lysine	AAA, AAG
L	Leu	Leucine	UUA, UUG, CUA, CUC, CUG, CUU
M	Met	Methionine	AUG
N	Asn	Asparagine	AAC, AAU
P	Pro	Proline	CCA, CCC, CCG, CCU
Q	Gln	Glutamine	CAA, CAG
R	Arg	Arginine	AGA, AGG, CGA, CGC, CGG, CGU
S	Ser	Serine	AGC, AGU, UCA, UCC, UCG, UCU
T	Thr	Threonine	ACA, ACC, ACG, ACU
V	Val	Valine	GUA, GUC, GUG, GUU
W	Trp	Tryptophan	UGG
Y	Tyr	Tyrosine	UAC, UAU

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A CDR mimetibody or specified portion or variant of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for at least one of a CDR mimetibody LBR, variable, constant, light or heavy chain, or Ig will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 amino acids, such as 1-30 or any range or value therein, as specified herein.

Amino acids in a CDR mimetibody or specified portion or variant of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at

every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one protein related activity, as specified herein or as known in the art. Sites that are critical for CDR mimetibody or specified portion or variant binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

Mimetibodies or specified portions or variants of the present invention can comprise, but are not limited to, at least one portion, sequence or combination selected from 3 to all the of at least one of SEQID NOS:1-1109.

Non-limiting variants that can enhance or maintain at least one of the listed activities include, but are not limited to, any of the above polypeptides, further comprising at least one mutation corresponding to at least one substitution, insertion or deletion that does not significantly affect the suitable biological activities or functions of said CDR mimetibody.

A(n) CDR mimetibody or specified portion or variant can further optionally comprise at least one functional portion of at least one polypeptide of at least one of 90-100% of SEQID NOS:1-1109.

A CDR mimetibody can further optionally comprise an amino acid sequence selected from one or more of SEQID NOS:1-1109.

In one embodiment, the amino acid sequence of an immunoglobulin chain, or portion thereof (e.g., comprising at least one specified variable region, LBR) has about 90-100% identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the corresponding amino acid sequence of the corresponding portion of at least one of SEQID NOS: 1-1109. Preferably, 90-100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

Mimetibodies or specified portions or variants of the present invention can comprise any number of contiguous amino acid residues from a CDR mimetibody or specified portion or variant of the present invention, wherein that number is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in a CDR mimetibody or specified portion or variant, including specified LBR sequences. Optionally, this subsequence of contiguous amino acids is at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more.

As those of skill will appreciate, the present invention includes at least one biologically active CDR mimetibody or specified portion or variant of the present invention. Biologically active mimetibodies or specified portions or variants have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous or related and known inserted or fused protein or specified portion or variant. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to human mimetibodies and ligand-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce a CDR mimetibody or ligand-binding fragment with improved pharmacokinetic properties (e.g., increased *in vivo* serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

The modified mimetibodies and ligand-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the CDR mimetibody or specified portion or variant. Each organic moiety that is bonded to a CDR mimetibody or ligand-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, a CDR mimetibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying mimetibodies of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the CDR mimetibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example, PEG<sub>2500</sub>, PEG<sub>5000</sub>, PEG<sub>7500</sub>, PEG<sub>9000</sub>, PEG<sub>10000</sub>, PEG<sub>12500</sub>, PEG<sub>15000</sub>, and PEG<sub>20,000</sub>, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used.

The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or

fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying mimetibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying mimetibodies of the invention include, for example, n-dodecanoate (C<sub>12</sub>, laurate), n-tetradecanoate (C<sub>14</sub>, myristate), n-octadecanoate (C<sub>18</sub>, stearate), n-eicosanoate (C<sub>20</sub>, arachidate), n-docosanoate (C<sub>22</sub>, behenate), n-triacontanoate (C<sub>30</sub>), n-tetracontanoate (C<sub>40</sub>), *cis*- $\Delta$ 9-octadecanoate (C<sub>18</sub>, oleate), all *cis*- $\Delta$ 5,8,11,14-eicosatetraenoate (C<sub>20</sub>, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human mimetibodies and ligand-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C<sub>1</sub>-C<sub>12</sub> group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, -(CH<sub>2</sub>)<sub>3</sub>-, -NH-(CH<sub>2</sub>)<sub>6</sub>-NH-, -(CH<sub>2</sub>)<sub>2</sub>-NH- and -CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CH<sub>2</sub>-NH-. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the

presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, *et al.*, WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified mimetibodies of the invention can be produced by reacting an human CDR mimetibody or ligand-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the CDR mimetibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human mimetibodies or ligand-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of a CDR mimetibody or ligand-binding fragment. The reduced CDR mimetibody or ligand-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified CDR mimetibody of the invention. Modified human mimetibodies and ligand-binding fragments comprising an organic moiety that is bonded to specific sites of a CDR mimetibody or specified portion or variant of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch *et al.*, *Bioconjugate Chem.*, 3:147-153 (1992); Werlen *et al.*, *Bioconjugate Chem.*, 5:411-417 (1994); Kumaran *et al.*, *Protein Sci.* 6(10):2233-2241 (1997); Itoh *et al.*, *Bioorg. Chem.*, 24(1): 59-68 (1996); Capellas *et al.*, *Biotechnol. Bioeng.*, 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996).

#### **CDR MIMETIBODY OR SPECIFIED PORTION OR VARIANT COMPOSITIONS**

The present invention also provides at least one CDR mimetibody or specified portion or variant composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more mimetibodies or specified portions or variants thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the CDR mimetibody amino acid sequence selected from the group consisting of 90-100% of the contiguous amino acids of SEQID NO:1-1109 or specified fragments, domains or variants thereof. Preferred CDR mimetibody compositions include at least one or two full length, fragments, domains or variants as at least one of the C-terminal amino acids of the protein sequence of 90-100% of SEQID NOS: 1-1109, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40-99% of at least one of 90-100% of SEQID NOS:1-1109 or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.



CDR mimetibody or specified portion or variant compositions of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the CDR mimetibody composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/CDR mimetibody or specified portion or variant components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

CDR mimetibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, the CDR mimetibody or specified portion or variant compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl- $\beta$ -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants

(e.g., polysorbates such as “TWEEN 20” and “TWEEN 80”), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the CDR mimetibody compositions according to the invention are known in the art, e.g., as listed in “Remington: The Science & Practice of Pharmacy”, 19<sup>th</sup> ed., Williams & Williams, (1995), and in the “Physician’s Desk Reference”, 52<sup>nd</sup> ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

### Formulations

As noted above, the invention provides for stable formulations, which can preferably include a suitable buffer with saline or a chosen salt, as well as optional preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one CDR mimetibody or specified portion or variant in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one CDR mimetibody or specified portion or variant with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one CDR mimetibody or specified portion or variant, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material

comprises a label that instructs a patient to reconstitute the at least one CDR mimetibody or specified portion or variant in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one CDR mimetibody or specified portion or variant used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

The range of amounts of at least one CDR mimetibody or specified portion or variant in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 µg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block copolymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic

container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one CDR mimetibody or specified portion or variant and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one CDR mimetibody or specified portion or variant and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one CDR mimetibody or specified portion or variant in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one CDR mimetibody or specified portion or variant that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biological activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to at least one of 1-12 months, one-half, one and a half, and/or two years.

The solutions of at least one CDR mimetibody or specified portion or variant in the invention can be prepared by a process that comprises mixing at least one CDR mimetibody or specified portion or variant in an aqueous diluent. Mixing is carried out using conventional dissolution

and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one CDR mimetibody or specified portion or variant in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and means of administration used.

The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one CDR mimetibody or specified portion or variant that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one CDR mimetibody or specified portion or variant that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one CDR mimetibody or specified portion or variant solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as Humaject<sup>®</sup>, NovoPen<sup>®</sup>, B-D<sup>®</sup>Pen, AutoPen<sup>®</sup>, and OptiPen<sup>®</sup>. Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen<sup>®</sup>.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one CDR mimetibody or specified portion or variant in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one CDR mimetibody or specified portion or variant and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one CDR mimetibody or

specified portion or variant and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one CDR mimetibody or specified portion or variant in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the  
5 desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear  
10 solutions or as dual vials comprising a vial of lyophilized at least one CDR mimetibody or specified portion or variant that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

15 At least one CDR mimetibody or specified portion or variant in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

## 20 **Therapeutic Applications**

The present invention for mimetibodies also provides a method for modulating or treating anemia, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of any  
25 anemia, cancer treatment related anemia, radiotherapy or chemotherapy related anemia, viral or bacterial infection treatment related anemia, renal anemia, anemia of prematurity, pediatric and/or adult cancer-associated anemia, anemia associated with lymphoma, myeloma, multiple myeloma, AIDS-associated anemia, concomitant treatment for patients with or without autologous blood donation awaiting elective surgery, preoperative and post operative for surgery, autologous blood donation or  
30 transfusion, perioperative management, cyclic neutropenia or Kostmann syndrome (congenital agranulocytosis), end-stage renal disease, anemia associated with dialysis, chronic renal insufficiency, primary hemopoietic diseases, such as congenital hypoplastic anemia, thalassemia major, or sickle cell disease, vaso-occlusive complications of sickle cell disease. Furman et al., Pediatrics 1992; 90: 716-728, Goldberg Science. 1988;242:1412-1415; Paul et al., Exp Hematol. 1984;12:825-830; Erslev et al.,  
35 Arch Intern Med. 1968;122:230-235; Ersley et al., Ann Clin Lab Sci. 1980;10:250-257; Jacobs et al., Nature. 1985;313:806-810; Lin et al., Proc Natl Acad Sci USA. 1985;82:7580-7584; Law et al., Proc

Natl Acad Sci USA. 1986;83:6920-6924; Goldwasser et al., J Biol Chem. 1974;249:4202-4206; Eaves et al., Blood. 1978;52:1196-1210; Sawyer et al., Blood. 1989;74:103-109; Winearls et al., Lancet. 1986;2:1175-1178; Eschbach et al., N Engl J Med. 1987;316:73-78; Eschbach et al., Ann Intern Med. 1989;111:992-1000, each reference entirely incorporated herein by reference.

5 Mimetibodies of the present invention can also be used for non-renal forms of anemia induced, for example, by chronic infections, inflammatory processes, radiation therapy, and cytostatic drug treatment, and encouraging results in patients with non-renal anemia have been reported. See, e.g., Abels RI and Rudnick SA Erythropoietin: evolving clinical applications. *Experimental Hematology*, 19: 842-50 (1991); Graber SE and Krantz SB Erythropoietin: biology and clinical use. *Hematology/Oncol.*  
 10 *Clin. North Amer.* 3: 369-400 (1989); Jelkman W and Gross AJ (eds) Erythropoietin. Springer, Berlin 1989; Koury MJ and Bondurant MC The molecular mechanism of erythropoietin action. *European Journal of Biochemistry* 210: 649-63 (1992); Krantz SB Erythropoietin. *Blood* 77: 419-34 (1991); Tabbara IA Erythropoietin. Biology and clinical applications. *Archives of Internal Medicine* 153: 298-304 (1993), each entirely incorporated herein by reference.

15 The present invention also provides a method for modulating or treating an anemia or blood cell related condition, in a cell, tissue, organ, animal, or patient, wherein said anemia or blood cell related condition is associated with at least one including, but not limited to, at least one of immune related disease, cardiovascular disease, infectious, malignant and/or neurologic disease. Such a method can optionally comprise administering an effective amount of at least one composition or  
 20 pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating cancer/infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral  
 25 and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis, septic arthritis, peritonitis, pneumonia, epiglottitis, e. coli 0157:h7, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis/epididymitis,  
 30 legionella, lyme disease, influenza a, epstein-barr virus, vital-associated hemaphagocytic syndrome, vital encephalitis/aseptic meningitis, and the like; (ii) leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-hodgkin's lymphoma, Burkitt's  
 35 lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma,

nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, adenocarcinomas, sarcomas, malignant melanoma, and the like; or (iii) neurodegenerative diseases, multiple sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranuclear Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoproteinemia, ataxia, telangiectasia, and mitochondrial multi-system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit' such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerorden-Spatz disease; and Dementia pugilistica, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. See, e.g., the Merck Manual, 16<sup>th</sup> Edition, Merck & Company, Rahway, NJ (1992)

Such a method can optionally comprise administering an effective amount of at least one composition or pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, diabetic atherosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrhythmias, ventricular fibrillation, His bundle arrhythmias,



atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aortic and peripheral aneurysms, aortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive arterial disorders, peripheral atherosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphedema, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one CDR mimetibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluroquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a

cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2<sup>nd</sup> Edition, Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

5 Mimetibodies can also be used *ex vivo*, such as in autologous marrow culture. Briefly, bone marrow is removed from a patient prior to chemotherapy and treated with TPO and/or EPO, optionally in combination with mimetibodies, optionally in combination with one or more additional cytokines. The treated marrow is then returned to the patient after chemotherapy to speed the recovery of the marrow. In addition, TPO, alone and in combination with EPO mimetibodies and/or EPO, can also be  
10 used for the *ex vivo* expansion of marrow or peripheral blood progenitor (PBPC) cells. Prior to chemotherapy treatment, marrow can be stimulated with stem cell factor (SCF) or G-CSF to release early progenitor cells into peripheral circulation. These progenitors are optionally collected and concentrated from peripheral blood and then treated in culture with TPO and mimetibodies, optionally in combination with one or more other cytokines, including but not limited to SCF, G-CSF, IL-3, GM-  
15 CSF, IL-6 or IL-11, to differentiate and proliferate into high-density megakaryocyte cultures, which are optionally then be returned to the patient following high-dose chemotherapy. Doses of TPO for *ex vivo* treatment of bone marrow will be in the range of 100 pg/ml to 10 ng/ml, preferably 500 pg/ml to 3 ng/ml. Doses of mimetibodies will be equivalent in activity to EPO which can be used from 0.1 units/ml to 20 units/ml, preferably from 0.5 units/ml to 2 units/ml, or any range or value therein.

20 TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, ligand-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as  
25 thalidomide, tenidap, phosphodiesterase inhibitors (e.g, pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g.,  
30 captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNF $\alpha$  antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNF $\alpha$  activity *in vitro*, *in situ* and/or preferably *in vivo*. For example, a suitable TNF human antibody of the present invention

can bind TNF $\alpha$  and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNF $\alpha$ . A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNF $\alpha$  IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNF $\alpha$  in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNF $\alpha$ , the affinity constant of chimeric antibody cA2 was calculated to be  $1.04 \times 10^{10} \text{M}^{-1}$ . Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Colligan *et al.*, eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-2000); Kozbor *et al.*, *Immunol. Today*, 4:72-79 (1983); Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987-2000); and Muller, *Meth. Enzymol.*, 92:589-601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.

Additional examples of monoclonal anti-TNF antibodies that can be used in the present invention are described in the art (see, e.g., U.S. Patent No. 5,231,024; Möller, A. *et al.*, *Cytokine* 2(3):162-169 (1990); U.S. Application No. 07/943,852 (filed September 11, 1992); Rathjen *et al.*, International Publication No. WO 91/02078 (published February 21, 1991); Rubin *et al.*, EPO Patent Publication No. 0 218 868 (published April 22, 1987); Yone *et al.*, EPO Patent Publication No. 0 288 088 (October 26, 1988); Liang, *et al.*, *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, *et al.*, *Hybridoma* 6:305-311 (1987); Fendly *et al.*, *Hybridoma* 6:359-369 (1987); Bringman, *et al.*, *Hybridoma* 6:489-507 (1987); and Hirai, *et al.*, *J. Immunol. Meth.* 96:57-62 (1987), which references are entirely incorporated herein by reference).

TNF Receptor Molecules

Preferred TNF receptor molecules useful in the present invention are those that bind TNF $\alpha$  with high affinity (see, e.g., Feldmann *et al.*, International Publication No. WO 92/07076 (published April 30, 1992); Schall *et al.*, *Cell* 61:361-370 (1990); and Loetscher *et al.*, *Cell* 61:351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran *et al.*, *Eur. J. Biochem.* 223:831-840 (1994)), are also useful in the present invention. Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNF $\alpha$  inhibitory binding proteins (Engelmann, H. *et al.*, *J. Biol. Chem.* 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, may contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer *et al.*, *Eur. J. Immunol.* 21:2883-2886 (1991); Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Peppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991); Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Butler *et al.*, *Cytokine* 6(6):616-623 (1994); Baker *et al.*, *Eur. J. Immunol.* 24:2040-2048 (1994); Beutler *et al.*, U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor

fusion molecules can also be found in Capon *et al.*, U.S. Patent No. 5,116,964; Capon *et al.*, U.S. Patent No. 5,225,538; and Capon *et al.*, *Nature* 337:525-531 (1989), which references are entirely incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, New York (1987-2000).

Cytokines include, but are not limited to all known cytokines. See, e.g., CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

Any method of the present invention can comprise a method for treating a protein mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one CDR mimetibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one CDR mimetibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one other cytokines such as IL-3, -6 and -11; stem cell factor; G-CSF and GM-CSF. Within regimens of combination therapy, daily doses of other cytokines will in general be: GM-CSF, 5-15 .mu.g/kg; IL-3, 1-5 lg/kg; and G-CSF, 1-25 .mu.g/kg. Combination therapy with GM-CSF, for example, is indicated in patients with low neutrophil levels.

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one CDR mimetibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one CDR mimetibody or specified portion or variant /kilogram of

patient per dose, and preferably from at least about 0.1 to 100 milligrams CDR mimetibody or specified portion or variant /kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.1-5000  $\mu$ g/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses can optionally include 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and/or 30 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 14.9, 15.0, 15.5, 15.9, 16.0, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000  $\mu$ g/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one CDR mimetibody or specified portion or variant of the present invention 0.01 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or any combination thereof, using

single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.0001 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

For parenteral administration, the CDR mimetibody or specified portion or variant can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

### **Therapeutic Administration**

Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one CDR mimetibody or specified portion or variant according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

A CDR mimetibody of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

### **Parenteral Formulations and Administration**

Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or

semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

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### **Alternative Delivery**

The invention further relates to the administration of at least one CDR mimetibody or specified portion or variant by parenteral, subcutaneous, intramuscular, intravenous, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. Protein, CDR mimetibody or specified portion or variant compositions can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as creams and suppositories; for buccal, or sublingual administration particularly in the form of tablets or capsules; or intranasally particularly in the form of powders, nasal drops or aerosols or certain agents; or transdermally particularly in the form of a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

### **25 Pulmonary/Nasal Administration**

For pulmonary administration, preferably at least one CDR mimetibody or specified portion or variant composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one CDR mimetibody or specified portion or variant can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of CDR mimetibody or specified portion or variants are also known in the art. All such devices can use of formulations suitable for the administration for the dispensing of CDR mimetibody or specified portion or variant in an aerosol. Such aerosols can be comprised of either solutions (both



aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin<sup>®</sup> metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler<sup>™</sup> (Astra), Rotahaler<sup>®</sup> (Glaxo), Diskus<sup>®</sup> (Glaxo), Spiros<sup>™</sup> inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler<sup>®</sup> powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx<sup>™</sup> Aradigm, the Ultravent<sup>®</sup> nebulizer (Mallinckrodt), and the Acorn II<sup>®</sup> nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one CDR mimetibody or specified portion or variant is delivered by a dry powder inhaler or a sprayer. There are a several desirable features of an inhalation device for administering at least one CDR mimetibody or specified portion or variant of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10  $\mu\text{m}$ , preferably about 1-5  $\mu\text{m}$ , for good respirability.

#### **Administration of CDR mimetibody or specified portion or variant Compositions as a Spray**

A spray including CDR mimetibody or specified portion or variant composition protein can be produced by forcing a suspension or solution of at least one CDR mimetibody or specified portion or variant through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one CDR mimetibody or specified portion or variant composition protein delivered by a sprayer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

Formulations of at least one CDR mimetibody or specified portion or variant composition protein suitable for use with a sprayer typically include CDR mimetibody or specified portion or variant composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of at least one CDR mimetibody or specified portion or variant composition protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for

stabilization of the CDR mimetibody or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating CDR mimetibody or specified portion or variant composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating CDR mimetibody or specified portion or variant composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The CDR mimetibody or specified portion or variant composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the CDR mimetibody or specified portion or variant composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as mimetibodies, or specified portions or variants, can also be included in the formulation.

#### **Administration of CDR mimetibody or specified portion or variant compositions by a Nebulizer**

CDR mimetibody or specified portion or variant composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of CDR mimetibody or specified portion or variant composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of CDR mimetibody or specified portion or variant composition protein either directly or through a coupling fluid, creating an aerosol including the CDR mimetibody or specified portion or variant composition protein. Advantageously, particles of CDR mimetibody or specified portion or variant composition protein delivered by a nebulizer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

Formulations of at least one CDR mimetibody or specified portion or variant suitable for use with a nebulizer, either jet or ultrasonic, typically include CDR mimetibody or specified portion or variant composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of

at least one CDR mimetibody or specified portion or variant protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one CDR mimetibody or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one CDR mimetibody or specified portion or variant composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one CDR mimetibody or specified portion or variant include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one CDR mimetibody or specified portion or variant formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one CDR mimetibody or specified portion or variant caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as CDR mimetibody or specified portion or variant protein can also be included in the formulation.

#### **Administration of CDR mimetibody or specified portion or variant compositions By A Metered Dose Inhaler**

In a metered dose inhaler (MDI), a propellant, at least one CDR mimetibody or specified portion or variant, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10  $\mu\text{m}$ , preferably about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ . The desired aerosol particle size can be obtained by employing a formulation of CDR mimetibody or specified portion or variant composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one CDR mimetibody or specified portion or variant for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one CDR mimetibody or specified portion or variant as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a

hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one CDR mimetibody or  
5 specified portion or variant as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation.

10 One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one CDR mimetibody or specified portion or variant compositions via devices not described herein.

#### **Mucosal Formulations and Administration**

For absorption through mucosal surfaces, compositions and methods of administering at least  
15 one CDR mimetibody or specified portion or variant include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal,  
20 pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch,  
25 and the like (U.S. Pat. Nos. 5,849,695).

#### **Oral Formulations and Administration**

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic  
30 inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and  
35 glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent,

lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid  
5 preparations for oral administration include emulsion, syrup, elixir, suspension and solution  
preparations allowable for medical use. These preparations may contain inactive diluting agents  
ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems  
for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers  
of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No.  
10 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No.  
5,587,753 are used to deliver biologically active agents orally are known in the art.

#### **Transdermal Formulations and Administration**

For transdermal administration, the at least one CDR mimetibody or specified portion or  
variant is encapsulated in a delivery device such as a liposome or polymeric nanoparticles,  
15 microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless  
otherwise stated). A number of suitable devices are known, including microparticles made of synthetic  
polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof,  
polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen,  
polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations  
20 thereof (U.S. Pat. Nos. 5,814,599).

#### **Prolonged Administration and Formulations**

It can be sometimes desirable to deliver the compounds of the present invention to the subject  
over prolonged periods of time, for example, for periods of one week to one year from a single  
administration. Various slow release, depot or implant dosage forms can be utilized. For example, a  
25 dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low  
degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as  
phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid,  
polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a  
salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum,  
30 copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-  
dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate  
salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt  
such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel  
with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts,  
35 pamoate salts, and the like. Another type of slow release depot formulation for injection would contain

the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No.

3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow  
5 release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by  
10 reference to the following examples, which are provided by way of illustration and are not intended as limiting.

**Example 1: Cloning and Expression of EPO CDR mimetibody in Mammalian Cells**

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the CDR mimetibody or specified portion or variant coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human HeLa 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded CDR mimetibody or specified portion or variant. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., *Biochem. J.* 227:277-279 (1991); Bebbington, et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of CDR mimetibody or specified portion or variants.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., *Molec. Cell. Biol.* 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

**Cloning and Expression in CHO Cells**

The vector pC4 is used for the expression of CDR mimetibody or specified portion or variant. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the EPO in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.



The DNA sequence encoding the complete CDR mimetibody or specified portion or variant is used, e.g., as presented in SEQID NOS: 13, 14, 15, 16, 17, 18, corresponding to HC and LC variable regions of a CDR mimetibody of the present invention, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct (e.g., as provided in vector p1351).

The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5  $\mu$ g of the expression plasmid pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1  $\mu$ g/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1  $\mu$ g/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### **Example 2: Exemplary CDR Mimetibodies**

**Background.** Erythropoietin has a relatively short half-life. Intravenously administered erythropoietin is eliminated at a rate consistent with first order kinetics with a circulating half-life ranging from approximately 3 to 4 hours in patients with CRF. Within the therapeutic dose range, detectable levels of plasma erythropoietin are maintained for at least 24 hours. After subcutaneous administration of erythropoietin, peak serum levels are achieved within 5-24 hours and decline slowly thereafter. The C max and t  $\frac{1}{2}$  after administration of erythropoietin were  $1.80 \pm 0.7$  U/mL and  $19.0 \pm 5.9$  hours, respectively.

Starting doses of erythropoietin range from 50-150 U/kg three times weekly. The dosage of erythropoietin must be individualized to maintain the hematocrit within the suggested target range. For surgery patients the recommended dose of erythropoietin is 300 U/kg/day s.c. for 10 days before

surgery, on the day of surgery, and for 4 days after surgery or alternatively 600 U/kg s.c. in once weekly doses (21, 14 and 7 days before surgery) plus a fourth dose on the day of surgery.

The 1998 world wide market size for therapeutic proteins was \$16.6B, of which the largest segment was \$3.6B (21%) for erythropoietin. Erythropoietin is used to treat anemia. The market is large, partly because the disorder can result from other kinds of diseases, such as cancer or renal dialysis.

Small peptidomimetics of erythropoietin were identified by several groups through screening of random phage display peptide libraries for affinity to the erythropoietin receptor. These sequences have no homology with erythropoietin. In functional assays several of these peptides showed activity, but only 1/100,000<sup>th</sup> that of recombinant erythropoietin. The consensus sequences YXCXXGPXTWXCXP (SEQ ID NO:980) and CXXGWVGXCXXW (SEQ ID NO:981) that have no homology with erythropoietin were identified based solely on receptor binding. One group has claimed peptides of the motif XXXGPXTWXX (SEQ ID NO:982) that are erythropoietin agonists while another group claims peptides of the motif XXXGPXTWXX (SEQ ID NO:983) that, when dimeric, activate the erythropoietin receptor but that are inactive as monomers.

Although several attempts have been made to increase the potency of these peptides by preparing covalent dimers or multimers of peptidomimetics, these compounds are still 1,000 - 10,000 fold less active than erythropoietin on a molar basis.

Peptide sequences from erythropoietin have also been claimed as agonistic. Increased activity of dimerized sequences comprising any or all of the native erythropoietin sequence has also been reported.

These compounds have little or no oral bioavailability and their activity does not make them economically viable at this time.

The invention provides for the design, production and use of erythropoietic mimetibodies, compounds that support peptidomimetics on an antibody scaffold. It is anticipated that these compounds will have erythropoietic activity similar to recombinant or native erythropoietin or analogs thereof, but will be superior because of extended half-life. This will permit sustained activity with less frequent dosing.

Using molecular modeling, a germline antibody sequence and the coordinates of the EMP-1/EPO receptor crystal structure, six CDR mimetibody structures were designed. The germline antibody sequence is used merely to illustrate the concept. It is expected that any number of other antibodies of any isotype can be used. Likewise, although the structure of EMP-1 is used to create an erythropoietic CDR mimetibody, it is expected that any number of peptidomimetic structures can be substituted to create active mimetibodies. While the concept is designed to work with peptidomimetics of proteins that cause receptor multimerization that lead to activity, monomeric binding leading to receptor activation is

envisioned as well. The six mimetibodies have the peptides attached to six different combinations of CDRs. Other constructs using different combinations of CDRs, different amino acids as attachment points for grafting the peptidomimetics onto the CDRs and different lengths and compositions of joining regions between the peptidomimetics and the antibodies can be used as well.

- 5 The sequences of the six example mimetibodies aligned with the germline antibody sequence are shown below.

Table 18: (SEQ ID NOS:984-990)

10 Light Chain

	10	20	30	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM1. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM2. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM3. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM4. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM5. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-EM6. PRO	
1	EIVLTQSPATLSLSPGERATLS	CRASQSSCTYSCHFGPL	L-SYNTH. PRO	
	40	50	60	
41	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM1. PRO	
39	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM2. PRO	
38	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM3. PRO	
31	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM4. PRO	
31	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM5. PRO	
31	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-EM6. PRO	
31	WVCKPQGS	SYLA WYQOKPGQAPRLLIYDASNRATGIP	L-SYNTH. PRO	
	70	80	90	
81	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM1. PRO	
78	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM2. PRO	
76	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM3. PRO	
61	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM4. PRO	
61	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM5. PRO	
61	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-EM6. PRO	
61	RFSGSGSGTDFTLTIS	SLEPEDFAVYYCQQRN	L-SYNTH. PRO	
	100			
114	WVCKPQGS	PPFTFGPGTKVDIK	L-EM1. PRO	
116	WVCKPQGS	PPFTFGPGTKVDIK	L-EM2. PRO	
111	WVCKPQGS	PPFTFGPGTKVDIK	L-EM3. PRO	
101	WVCKPQGS	PPFTFGPGTKVDIK	L-EM4. PRO	
99	WVCKPQGS	PPFTFGPGTKVDIK	L-EM5. PRO	
94	WVCKPQGS	PPFTFGPGTKVDIK	L-EM6. PRO	
94	WVCKPQGS	PPFTFGPGTKVDIK	L-SYNTH. PRO	

Decoration 'Decoration #1': Shade (with solid bright yellow) residues that differ from L-SYNTH. PRO.

Decoration 'Decoration #2': Box residues that differ from L-SYNTH. PRO.

Table 19: (SEQ ID NOS: 991-996)

## Heavy Chain

	10	20	30	
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-EM1.PRO
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-EM2.PRO
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-EM3.PRO
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-EM4.PRO
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-EM5.PRO
1	Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S			H-SYNTH.PRO
	40	50		
31	S Y A M H W V R Q A P G K G L E W V A V I S			H-EM1.PRO
31	S Y A M H W V R Q A P G K G L E W V A V I S			H-EM2.PRO
31	S Y A M H W V R Q A P G K G L E W V A V I S	S G S G G T Y S C H		H-EM3.PRO
31	S Y A M H W V R Q A P G K G L E W V A V I S	E S E G T Y S C H		H-EM4.PRO
31	S Y A M H W V R Q A P G K G L E W V A V I S			H-EM5.PRO
31	S Y A M H W V R Q A P G K G L E W V A V I S			H-SYNTH.PRO
	60	70		
54		D G S N K Y Y A D S V K D R F T I S R D		H-EM1.PRO
54		D G S N K Y Y A D S V K D R F T I S R D		H-EM2.PRO
61	F G P L T W V C K P Q G	G S G S T A D S V K D R F T I S R D		H-EM3.PRO
61	F G P L T W V C K P Q G	G K - - Y A D S V K D R F T I S R D		H-EM4.PRO
54		D G S N K Y Y A D S V K D R F T I S R D		H-EM5.PRO
54		D G S N K Y Y A D S V K D R F T I S R D		H-SYNTH.PRO
	80	90	100	
74	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I S			H-EM1.PRO
74	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I G			H-EM2.PRO
91	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I S			H-EM3.PRO
89	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I G			H-EM4.PRO
74	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I - E			H-EM5.PRO
74	N S K N T L Y L Q M N S L R A E D T A V Y Y C A R D R G I G			H-SYNTH.PRO
		110		
104	G G G G T Y S C H F G P L T W V C K P Q G G Y Y Y Y G M D V			H-EM1.PRO
104	G - - - - - - - - - - W - - - - - Q N Y Y Y Y G M D V			H-EM2.PRO
121	S P - - - - - - - - - - - - - - E - - - - - Y Y G M D V			H-EM3.PRO
119	G W - - - - - - - - - - - - - - Q N Y Y Y Y G M D V			H-EM4.PRO
103	G S G G T Y S C H F G P L T W V C K P Q G G G S G - S M D V			H-EM5.PRO
104	G - - - - - - - - - - - - - - W - - - - - Q N Y Y Y Y G M D V			H-SYNTH.PRO

5

The three dimensional structures of the molecular models of the variable regions of the six structures were calculated and plotted and determined to provide suitable presentation of the exemplary therapeutic proteins and polypeptides for therapeutic activity. Immunoglobulins are known to have long half-lives as compared to both peptide and non-peptide drugs. The joining of two EMP-like peptides to the CDRs of an immunoglobulin provides the necessary structure for receptor dimerization and is expected to have a half-life that will allow for dosing only 1-2 times per month or less. Given the extended half-life smaller amounts of material may be required for similar erythropoietic activity.

10

It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the  
5 above teachings and, therefore, are within the scope of the appended claims.

## WHAT IS CLAIMED IS:

1. A CDR mimetibody, comprising at least one portion of a heavy chain or light chain variable region comprised of at least one human framework region and at least one ligand binding region (LBR) comprised of at least one biologically active polypeptide, which LBR further comprises at least one amino acid from at least one complementarity determining region (CDR) of a heavy or light chain variable region, wherein said biologically active polypeptide is selected from at least one of SEQ ID NOS:1-1109.
2. A CDR mimetibody according to claim 1, wherein said at least one CDR is CDR1, CDR2 or CDR3 of the heavy or light chain variable region and said at least one human framework region is FR1, FR2 or FR3 of the heavy or light chain variable region.
3. A CDR mimetibody according to claim 1, wherein said at least one CDR amino acid sequence optionally further comprises at least one specified substitution, insertion or deletion.
4. A composition comprising at least one CDR mimetibody according to claim 1 and a suitable carrier or diluent.
5. A composition according to claim 4, wherein the carrier or diluent is pharmaceutically acceptable.
6. A composition according to claim 4, wherein said composition further comprises at least one further compound, protein or composition.
7. A method for providing at least one CDR mimetibody according to claim 1 in a host cell, comprising culturing a host cell under conditions wherein at least one CDR mimetibody is expressed in detectable and/or recoverable amounts.
8. A method for treating a biologically active protein related pathology, comprising  
(a) administering a therapeutically effective amount of at least one CDR mimetibody according to claim 1.
9. A method according to claim 8, wherein the pathology is selected from at least one of a(n) anemia condition; a(n) immune/autoimmune condition; and/or a(n) cancer/infectious condition.
10. The present invention further provides at least one CDR mimetibody, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of, at least one immune, cardiovascular, infectious, malignant, and/or

neurologic disease in a cell, tissue, organ, animal or patient and/or, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art and/or as described herein.

- 5           11. An anti-idiotypic antibody that specifically binds at least one CDR mimetibody according to claim 1.
12. An isolated nucleic acid encoding an anti-idio-type antibody according to claim 11.
13. A nucleic acid vector, comprising an isolated nucleic acid according to claim 12.
14. A host cell comprising a nucleic acid according to claim 13.
- 10          15. A CDR mimetibody according to claim 1, wherein the CDR region is derived from a mammal.
16. A CDR mimetibody according to claim 12, wherein the mammal is selected from a human, a mouse, a rabbit, a rat, a rodent, or a primate.
17. A CDR mimetibody isolated nucleic acid molecule, comprising, complementary, or hybridizing to, a polynucleotide encoding at least one CDR mimetibody according to claim 1.
- 15           18. A nucleic acid vector, comprising an isolated nucleic acid according to claim 17.
19. A host cell comprising a nucleic acid according to claim 18.
20. A CDR mimetibody according to claim 1, wherein said LBR comprises at least one selected from (i) all of the heavy chain complementarity determining regions (CDR); or (ii) all of the light chain CDR amino acids sequences.
- 20           21. A CDR mimetibody according to claim 1, wherein said CDR mimetibody binds at least one ligand for said ligand binding region with an affinity of at least one selected from at least  $10^{-9}$  M, at least  $10^{-10}$  M, at least  $10^{-11}$  M, or at least  $10^{-12}$  M.
- 25           22. A CDR mimetibody according to claim 21, wherein said CDR mimetibody substantially neutralizes at least one activity of at least one of said ligand.
23. A host cell according to claim 19, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.
- 30           24. A method for producing at least one CDR mimetibody, comprising translating at least one CDR mimetibody nucleic acid according to claim 17, under conditions in vitro, in vivo or in situ, such that the CDR mimetibody is expressed in detectable or recoverable amounts.

25. A composition according to claim 6, wherein at least one further compound, protein or composition is selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
26. A method according to claim 8, wherein said effective amount is 0.001-50 mg/kilogram of the cells, tissue, organ or animal.
27. A method according to claim 8, wherein said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.
28. A method according to claim 8, further comprising administering, prior, concurrently or after the (a) administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
29. A medical device, comprising at least one isolated mammalian CDR mimetibody according to claim 1, wherein the device is suitable to contacting or administering the at least one CDR mimetibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial,



intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

30. An article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated mammalian CDR mimetibody according to claim 1.

31. An article of manufacture according to claim 30, wherein the article of manufacture further comprises at least one container as a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

32. A method for providing at least one isolated mammalian CDR mimetibody according to claim 1, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts the CDR mimetibody.

33. At least one CDR mimetibody produced by a method according to claim 32.

34. A CDR mimetibody according to claim 1, wherein said light chain variable region comprises at least one amino acid sequence selected from SEQ ID NOS:984-990.

35. A CDR mimetibody according to claim 1, wherein said heavy chain variable region comprises at least one amino acid sequence selected from SEQ ID NOS:991-996.